HIGHLY PATHOGENIC H5 AVIAN INFLUENZA IN THE AMERICAS

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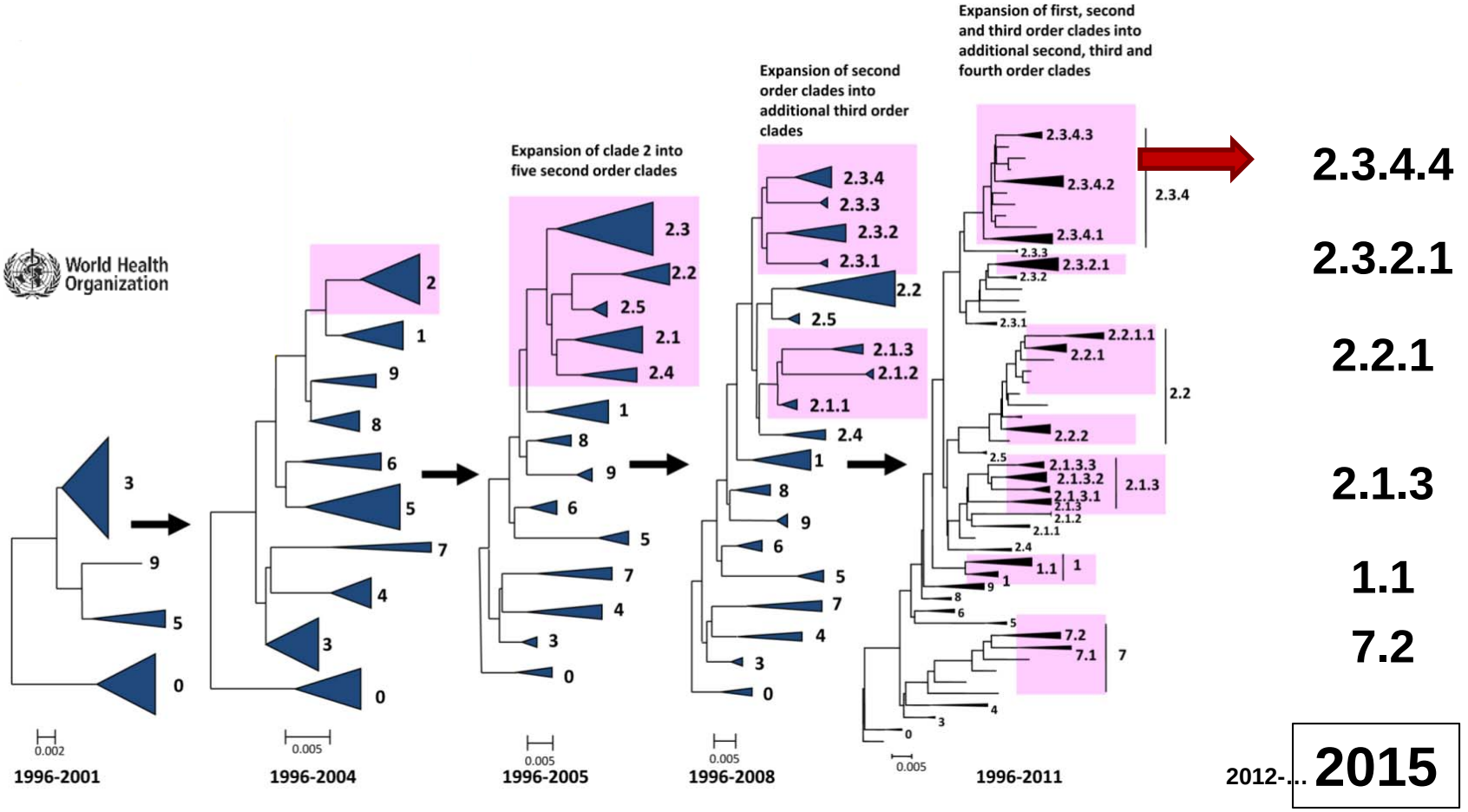
H5 Goose/Guangdong-lineage HPAIV



- **Since 1996 – H5N1 hemagglutinin changes – e.g. DRIFT (similar to human seasonal flu)**
- **Since 2008 – reassortment of NA genes (N5, N6, N8, N2, N3)**

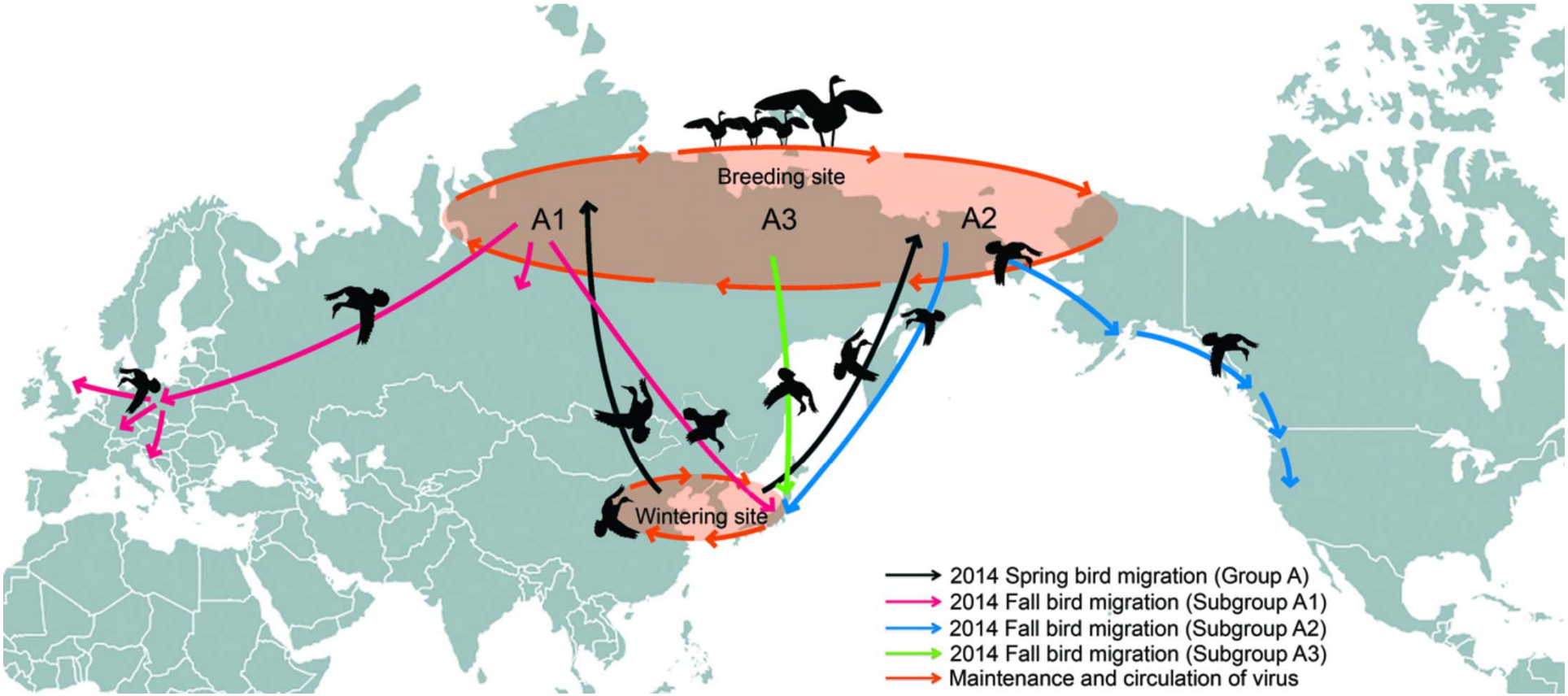
Origins H5N8 in Asia

- H5N8 Highly Pathogenic Avian Influenza was reported in South Korea in wild and domestic birds in early 2014 causing serious outbreaks in poultry
- Virus was reassortant with H5N1 goose/Guangdong lineage poultry virus and presumable a wild bird isolate
- HA cleavage site was typical of HPAI
- Isolates were classified as clade 2.3.4.4 viruses
- The H5N8 lineage of virus represents the third major spillover of HPAI from poultry to wild birds

H5N8 HPAIV and derivatives: Eurasia

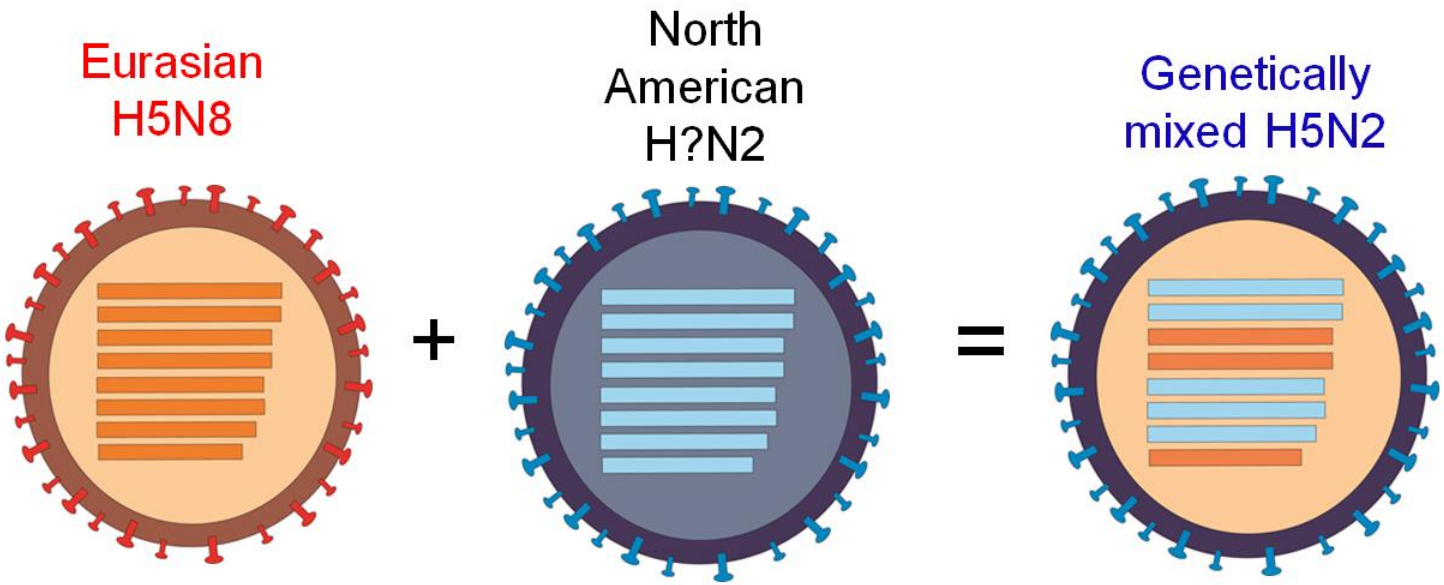
- South Korea
  - January 2014 reported in domestic birds
  - Numerous outbreaks through September 2014
- Japan
  - April 2014 H5N8
  - Outbreaks in chickens, swans cranes and wild ducks
- China
  - October 2014 H5N8
  - Environmental sample and duck sample during routine surveillance
- Russia
  - December 2014
  - Wild duck isolate
- Europe
  - November 2014-Feb 2015 in Germany, Netherlands, Italy, UK, Hungary
- Taiwan
  - January 2015 H5N8, H5N2, and H5N23 in poultry

Movement of H5N8 into North America



New Eurasian H5 viruses in North America

The wholly Eurasian H5N8 virus has mixed with North American avian influenza viruses, creating *new mixed-origin* viruses: H5N2 and H5N1



HPAI Outbreaks by County

- H5N2 detected in Minnesota on March 4, 2015
- Outbreaks in turkeys and layer farms continued through June 2015
- Outbreaks in poultry contained in June with no new detections of virus reported

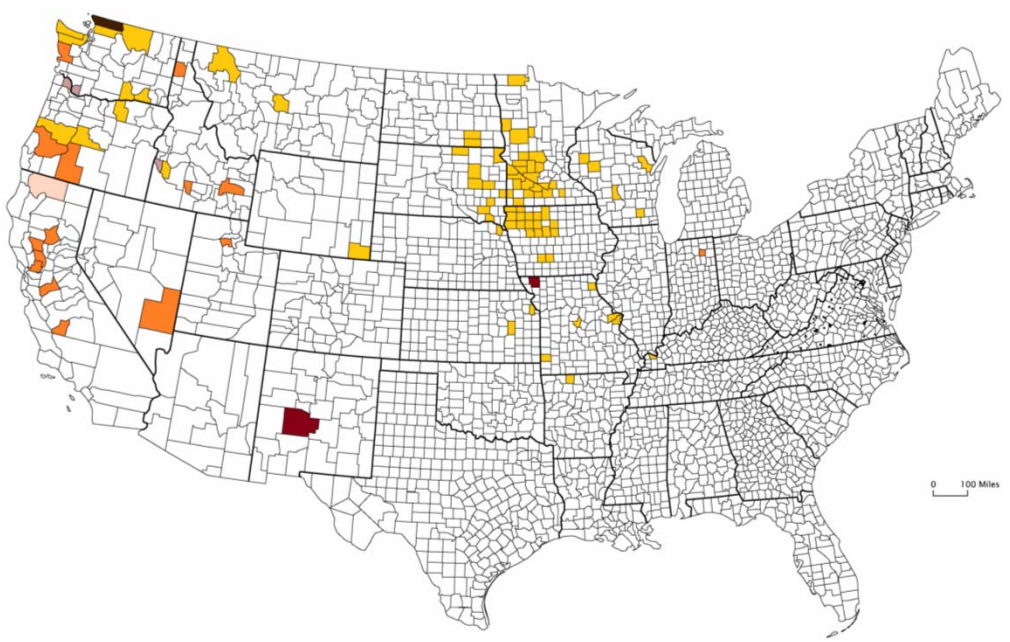
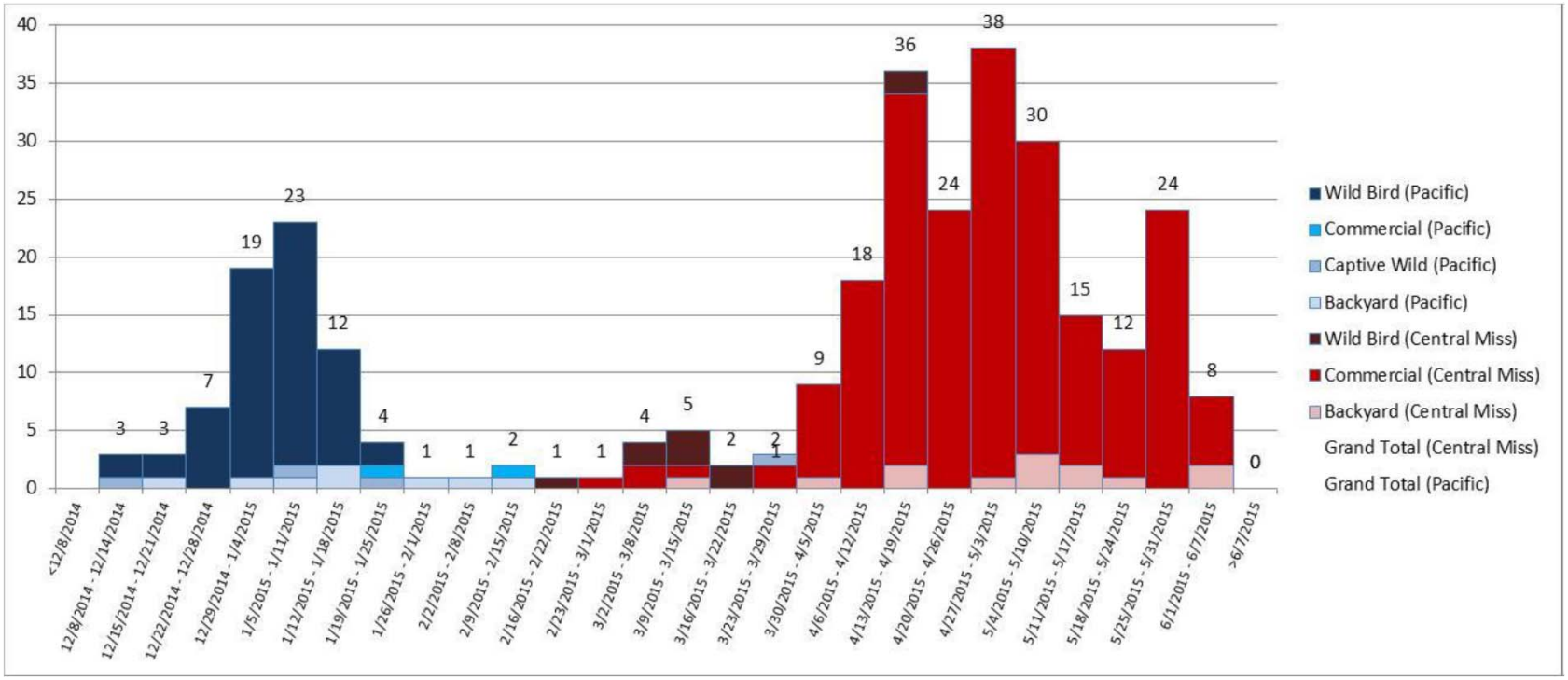


Figure 9. HPAI Detections *by Subtype in All Birds,* As Of June 5, 2015 PM (as reported on www.aphis.usda.g

H5N8 H5N2 H5N8 AND H5N2 H5N1, H5N8, AND H5N2 H5 (positive icA, no virus iso

H5N8, H5 (positive icA)

12/8/2015 to 6/12/2015 – H5 HPAIV in wild bird, backyard poultry and commercial poultry



- **309 detections (4 captive wild bird; 21 backyard; 209 commercial flocks, 75 wild birds)**
  - **21 states affected (AR , CA, IA, ID, IN, KS, KY, MI, MN, MO, MT, NE, ND, NM, NV, OR, SD, UT, WA, WI, WY)**
  - **~ 48.6 million commercial birds: Turkeys ~7.5 million, Chickens ~41.1 million**

Why is this outbreak so much different than previous outbreaks

- Wild bird reservoir
  - First time in U.S. dealing with wild bird reservoir of HPAIV
  - The prevalence of infection in some wild duck species is higher than would normally be expected for a low pathogenic isolate
  - Allowed exposure of poultry to HPAIV in over 20 states
- Size of farms
  - Egg layer operations in midwest are typically a million+ bird operation and have lots of movement on and off the farms
  - Once virus introduced onto farm, the virus will adapt
- Logistical issues
  - Difficult to depopulate that large numbers of birds
  - Difficult to dispose of that large number of birds
  - Budget cuts at USDA reduced numbers of veterinarians

Public Health

- Currently no human cases of H5N8 or H5N2 viruses
- However, these viruses have genes from the Asian H5N1 lineage known to cause rare human infections, but with high case fatality rate
  - Human to human transmission extremely rare for H5N1 viruses
  - Unknown whether the genetic changes in these strains make them more or less of threat to human health
  - Initial studies in mammals show less virulence for H5N8 than H5N1 viruses
- Sensitive to Tamiflu and other antivirals based on *in vitro* testing
  - Treatment should be started as early as possible
- PPE needs to be used as if this were known to be human infectious

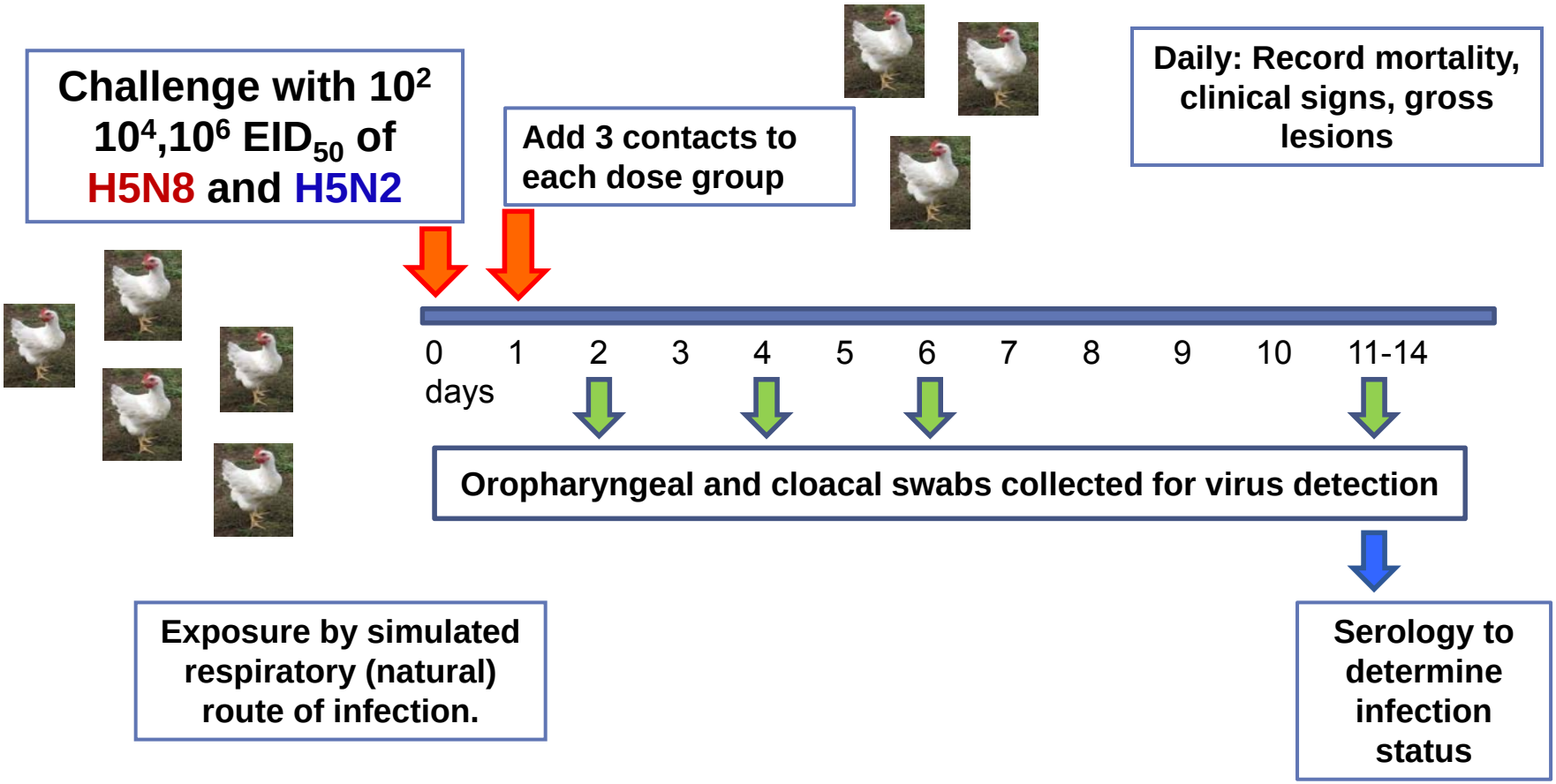
Pathogenesis and Transmission

Determine any differences in infectivity, transmissibility & pathogenesis of the intercontinental H5Nx clade 2.3.4.4 HPAIV for gallinaceous poultry and mallard ducks

Approach

- **Used two earliest USA isolates:**
  - **A/Gyrfalcon/Washington/41088/2014 (H5N8)**
  - **A/Northern Pintail/Washington/40964/2014 (H5N2)**
- **Intranasal infectious dose and contact transmission**
  - **SPF White Leghorn chickens**
  - **Commercial broad-breasted white turkeys**
  - **Commercial Japanese quail, pheasant, Bob-white quail**
  - **Mallard ducks**
- **Pathogenesis studies: preclinical, clinical & dead birds for virus shedding, histopathology and IHC-virus distribution**

Infectious dose and virus transmission in chickens, turkeys and quail



Virus detected by quantitative real-time RT-PCR assay and virus isolation

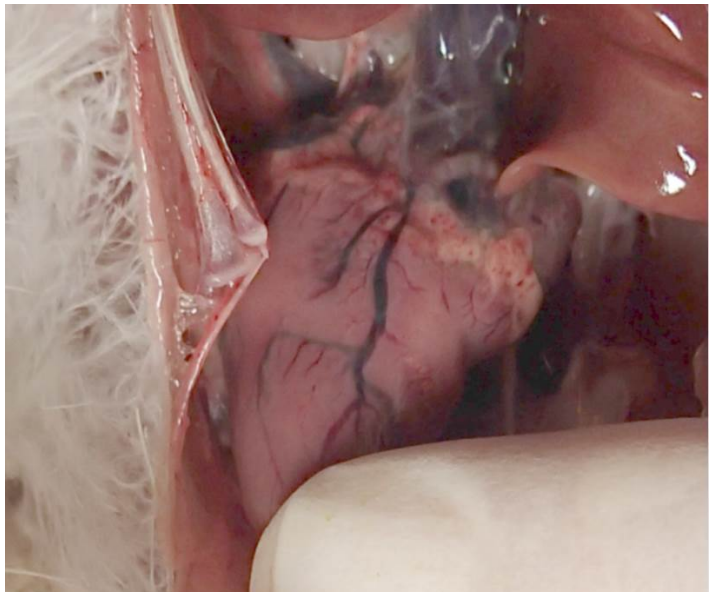
Chickens: Infectious dose and transmission

	Log 10 Dose	Inoculated dead/total (MDT)	Contact dead/total (MDT)	CLD50 Log 10
A/Gyrfalcon/Washington/41088/2014 H5N8 Dec 2014	2	0/5	0/3	
	4	2/5 (4d)	0/3	4.3
	6	5/5 (4.1d)	0/3	
A/Northern Pintail/Washington/40964/2014 H5N2 Dec 2014	2	0/5	0/3	
	4	0/5	0/3	5.7
	6	3/5 (3d)	0/3	
A/Tk/AR/7791/2015 H5N2 March 2015	2	0/5	0/3	
	4	0/5	0/3	5.1
	6	8/9 (2.3d)	0/2	
A/Tk/MN/12582/2015 H5N2 April 2015	2	0/5	0/3	
	4	3/5 (2d)	0/3	3.5
	6	8/8(2d)	2/2	

Pathogenesis: Chickens

- **Clinical Signs**
  - **Mild illness (~2-3d): ruffled feathers, listlessness, eyes partially closed**
  - **Severe illness including neurological signs by 4 DPI**
- **Mortality**
  - **100% in birds that were infected**
  - **MDT=3-4 Days**

- **Gross lesions**
  - **Not observed in all chickens**
  - **Necrotic combs/wattles, hemorrhages on shanks, necrotic pancreas, splenomegaly with pale color, petechial hemorrhages on the myocardium and skeletal muscle, enlarged pale kidneys, periorbital edema**



Turkeys: Infectious dose and transmission

- **H5N8: TLD_{50} = 4-6 (~5) log_{10}, 100% transmission to contacts at 6 log_{10} dose**
- **H5N2: TLD_{50} = 4-6 (~5) log_{10}, 100% transmission to contacts at 6 log_{10} dose**

	Log 10 Dose	Inoculated dead/total (MDT)	Contact dead/total (MDT)
A/Gyrfalcon/Washington/41088/2014 H5N8 Dec 2014	2	0/5	0/3
	4	0/5	0/3
	6	5/5 (9)	3/3 (12.5)
A/Northern Pintail/Washington/40964/2014 H5N2 Dec 2014	2	0/5	0/3
	4	0/5	0/3
	6	5/5 (5.3)	3/3(7.3)

Pathogenesis: Turkeys H5N2

- Susceptibility
  - Only those exposed to the highest dose were infected
  - 100% infection in contact transmission turkeys
- Mortality
  - 100%
  - MDT 5.3 days (long)
- Signs
  - Most had none until near death
    - Severe lethargy
    - Huddling, ruffled feathers
    - Neurological signs (torticollis)

	Log 10 Dose	Inoculated dead/total (MDT)	Contact dead/total (MDT)
H5N2	2	0/5	0/3
	4	0/5	0/3
	6	5/5 (5.3)	3/3 (7.3)


1976

Pathogenesis: Turkeys H5N8

- Susceptibility
  - Only those exposed to the highest dose were infected
  - 100% infection in contact transmission turkeys
- Mortality
  - 100%
  - MDT 9 days (very long)
- Signs
  - Disease not apparent until they are close to death
  - Severe lethargy, huddling, ruffled feathers
- Gross lesions
  - No lesions typically associated with AIV
- Virus shedding: pending

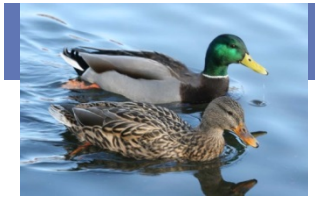
	Log 10 Dose	Inoculated dead/total (MDT)	Contact dead/total (MDT)
H5N8	2	0/5	0/3
	4	0/5	0/3
	6	5/5 (9)	3/3 (12.5)

Pathogenicity and transmission of H5N8 and H5N2 HPAI viruses in Mallards

Objective: To examine the pathogenicity (infectious dose, clinical signs, lesions, duration of virus shedding) and transmission of the H5N8 and H5N2 HPAIV's in mallards, comparing to an Asian lineage H5N1 HPAIV wild bird isolate and an American lineage LPAIV.

Study design:

- Two-week-old mallards (*Anas platyrhynchos*)
- Viruses:
  - A/GyrFalcon/Washington/41088/2014 (H5N8)
  - A/NPintail/Washington/40964/2014 (H5N2)
  - A/Whooper swan/Mongolia/244/2005 (H5N1)



Mallards - Infectious dose and transmission

	Log 10 Challenge Dose	Inoculated #pos/total	Mortality	Contact #pos/total	DLD50 Log 10
A/Gyrfalcon/Washington/41088/2014 H5N8 H5N8 Dec 2014	2	5/5	0/5	3/3	
	4	5/5	0/5	3/3	<2.0
	6	5/5	0/5	3/3	
	7.5	5/5	0/5	3/3	
A/Northern Pintail/Washington/40964/2014 H5N2 Dec 2014	2	5/5	0/5	3/3	
	4	5/5	0/5	3/3	<2.0
	6	5/5	0/5	3/3	
	7.5	5/5	0/5	3/3	
A/Whooper swan/Mongolia/244/2005 H5N1	6	10/10	10/10		

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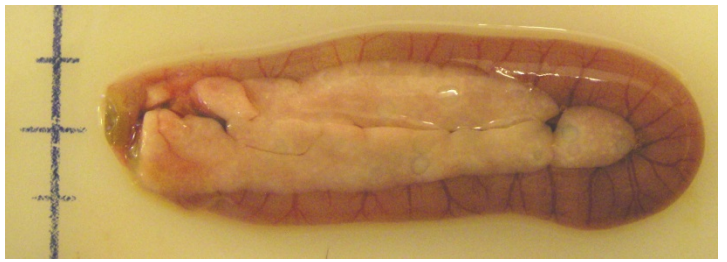
Japanese Quail: Infectious dose and transmission

- **H5N8: QLD_{50} = 2-4 (~3) log_{10}, no transmission to contacts**
- **H5N2: QLD_{50} = 3.6 log_{10}, 67% transmission to contacts at 6 log_{10} dose**

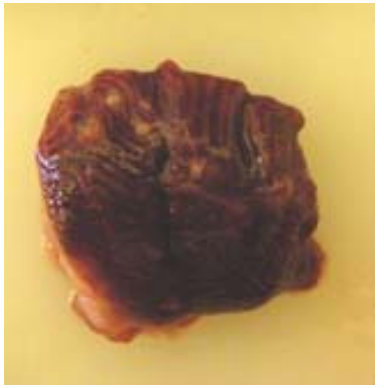
	Log 10 Dose	Inoculated dead/total (MDT)	Contact dead/total (MDT)
H5N8	2	0/5	0/3
	4	5/5 (2.5)	0/3
	6	4/5 (2.5)	0/3
H5N2	2	1/5 (3)	0/3
	4	3/5 (3)	0/3
	6	4/5 (2.75)	2/3

Pathogenesis: Japanese quail

- **Signs**
  - **Clinical signs – listlessness, ruffled feathers "sunk" eyes**
- **Mortality**
  - **100% in birds that were infected**
  - **MDT=2.5-3 Days**



- **Gross lesions**
  - **Not observed in all quail**
  - **Mildly sick birds: necrotic pancreas, hemorrhagic duodenal loop, splenomegaly and mottled spleen; petechial hemorrhages on the myocardium; pulmonary hemorrhage**
  - **Found dead birds: similar lesions as above but more pronounced plus petechial hemorrhages on the proventricular (glandular stomach) mucosa**



Conclusions

- **The two early 2.3.4.4 HPAIV strains were not well adapted to gallinaceous poultry**
  - **BID_{50}:100-1,000 times higher than previous H5N1 HPAIVs**
  - **Inefficient transmission to contacts except when placed with high challenge dose group**
- **Birds that became infected = died (BID_{50} = BLD_{50})**
- **Longer time to death than historic H5N1 HPAIV**
- **More restricted virus replication and lesions**
- **Susceptibility (including mallard study)**

  Mallards > Japanese Quail > Turkeys > Chickens
- **Later isolates were more adapted to chickens with lower BID_{50}**

Conclusions

- The H5Nx viruses were able to experimentally infect and transmit easily in mallard ducks with no clinical disease
- Supports the role of wild birds in moving the virus to North America
- Initial outbreaks in poultry in U.S. appear to be point source introductions with little farm to farm spread of virus
- Later outbreaks have evidence of farm to farm spread as major contributor to spread
- Control efforts in U.S. remain as early detection and stamping out of infected flocks
- Virus is evolving, likely in part to the high density of birds on the farms it has affected

Infectious dose and transmission - Original H5N2 and H5N8 viruses

Species	% Mortality	MDT (days)	BLD_{50} (log_{10})	Transmission to contacts
Chickens *(Gallus Gallus)*	60-100	3 - 4	4.3-5.7	No or only in 10^6 groups
Turkeys (*Meleagris gallopavo*)	100	5.3 - 9	5	Only in 10^6 groups
Japanese Quail (*Coturnix japonica)*	80	2.5 - 3	3.0 - 3.6	Only H5N2 in 10^6 group
Pheasants (*Phasianus colchicus*)	100	4.7 - 4.8	3.0 - 3.4	Yes, in 10^4 and 10^6 groups
Partridge (*Alectoris chukar)*	100	4.1 - 5.2	3.6	Yes, in 10^4 and 10^6 groups
Pekin ducks *(Anas platyrrhynchos var. dom.)*	0	-	$BID_{50} = 3\ log_{10}$	Yes, in 10^4 and 10^6 groups
White Chinese Geese (*Anser cygnoides*)	25	7-7.5	> 6	Yes
Mallards (*Anas platyrrhynchos*)	0	-	$BID_{50} \leq 2\ log_{10}$	Yes, in all groups

MDT=mean death time; BLD_{50}=Bird lethal dose $_{50}$

Other Research Activities

- Vaccines-covered by David Swayne
- Diagnostics
  - Real-time RT-PCR test specific for 2.3.4.4 lineage to provide rapid confirmation of HPAI infection
  - N2 antibody ELISA test to support DIVA vaccinations
- Wildbird surveillance
  - Sample testing from California and Alaska
- Network analysis of sequence information

Modifying C&D: on-farm inactivation of HPAIV

- Can we reduce time and cost of C&D by “cooking” the virus in the poultry house?
  - Similar to what we do for LT
  - Will test a range of temps and litter conditions
    - High temperatures not possible in colder regions
    - Does litter moisture affect time needed for inactivation
  - Develop inactivation curves
  - Aim to look at NDV as well
- Funded by USPEA and conducted by Spackman lab

Future

- Anticipate persistence of H5Nx HPAI in wild birds for at least 3-5 years
- Potential for introduction anywhere in the U.S.
- Increase in biosecurity and surveillance in poultry
- Increase surveillance in wild birds
- Increased resources to USDA to more quickly react to multiple outbreaks if they occur
- Research to better understand the virus and provide improved control tools

Contributors

- **SEPRL Avian Influenza Research Team: David Suarez, Mary Pantin-Jackwood, Erica Spackman, Darrell Kapczynski, David Swayne, Kateri Bertran, Mar Costa-Hurtado, Donghun Lee, Marisela Rodriguez, Yue Wang, Eric DeJesus, Charles Balzli, Kira Moresco, Diane Smith, Aniko Zsak, Scott Lee, Suzanne Deblois, Cam Greene, James Doster, Megan Christian, Nicolai Lee, Rebekah Lee, Samantha Pallas, Melissa Scott, Bill Gagnon, Roger Brock, Ronald Graham, Gerald Damron, Keith Crawford**

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