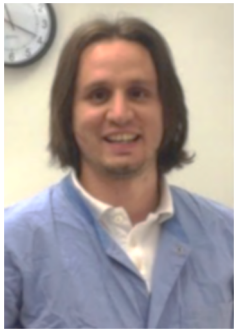
USDA Research Update

Team NADC

Daniela Rajao



Eugenio Abente

Rasna Walia

Not pictured:

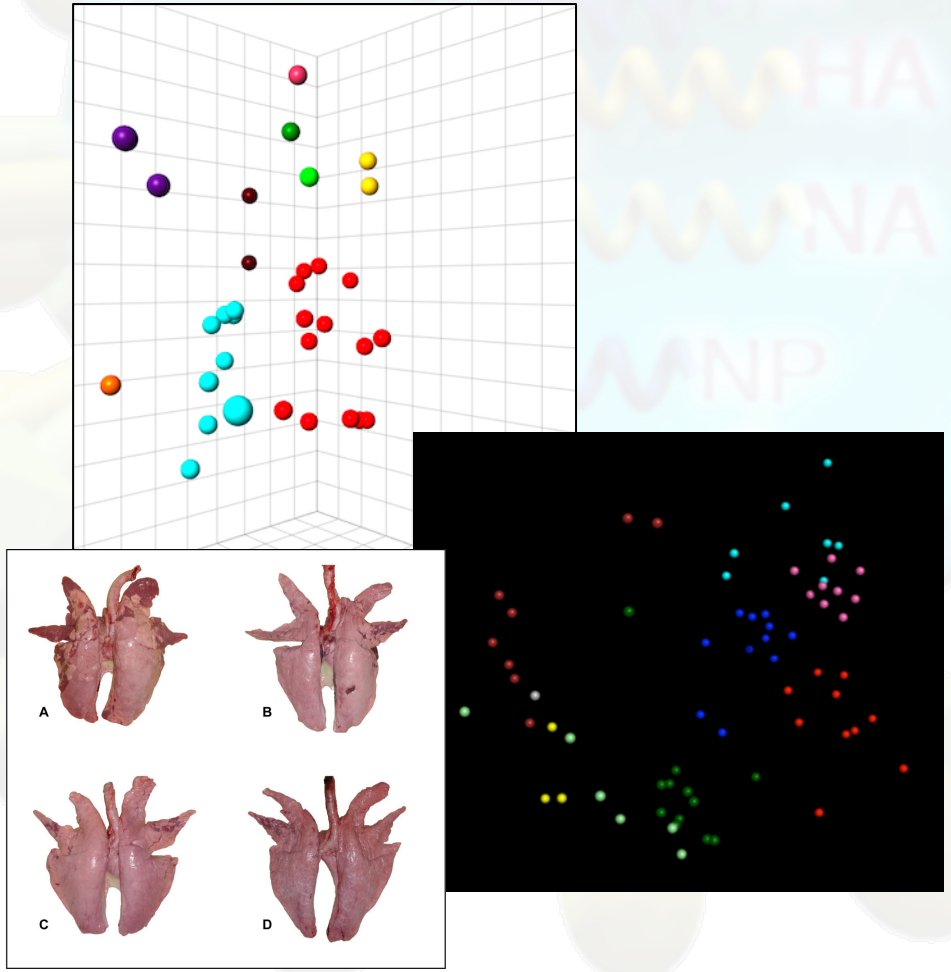
Tavis Anderson
Jered Stratton
Hylia Gao

USDA National Animal Disease Center
Agricultural Research Services
Ames, Iowa

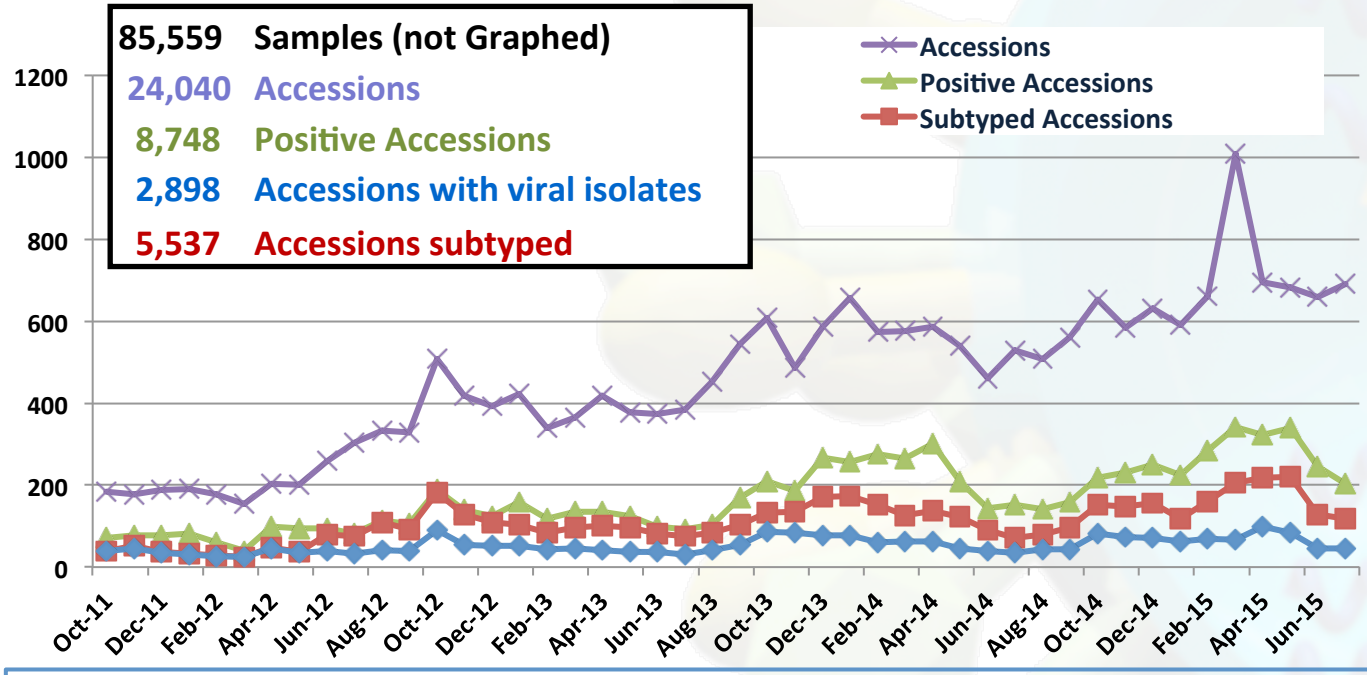
offlu
OIE FAO

NADC – Genetic and antigenic analyses and other *in vitro* and *in vivo* studies

- Phylogenetic and genetic evolution studies
- Isolates use to generate monovalent swine sera
  - Serum shared within CEIRS network for antigenic analyses
  - Cross-HI and antigenic cartography
  - Measure antigenic evolution and determinants
- Pathogenesis, transmission, and vaccine studies in the natural host



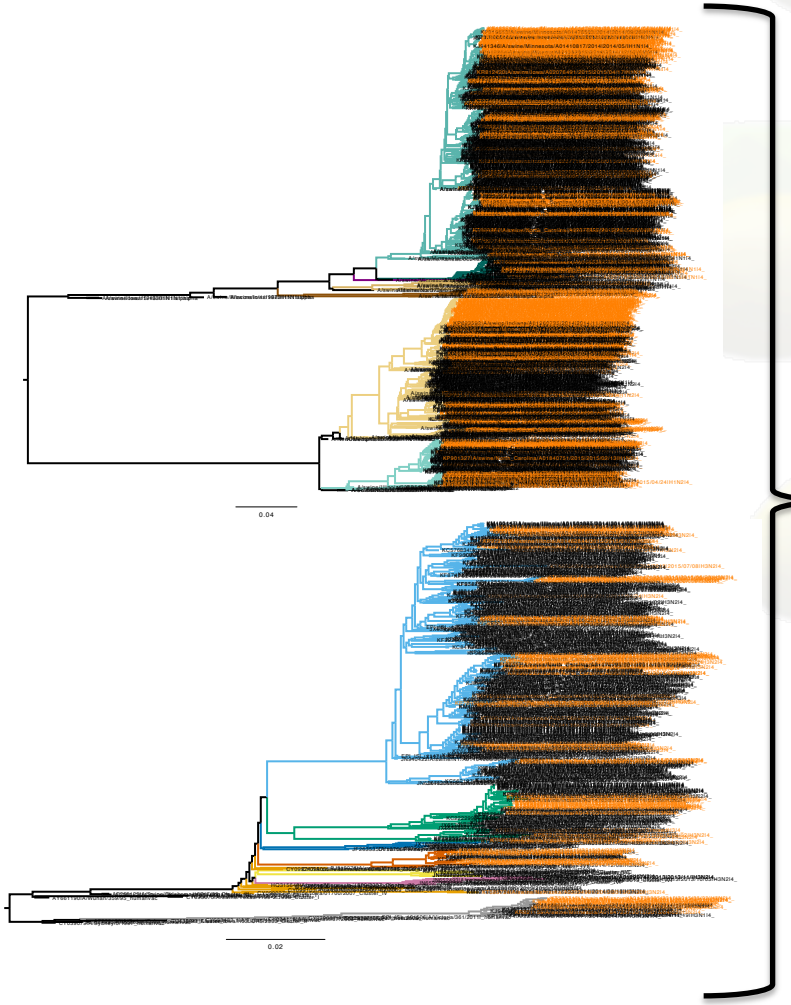
USDA IAV-S Surveillance
National Program Activities Oct. 2011 – June 2015

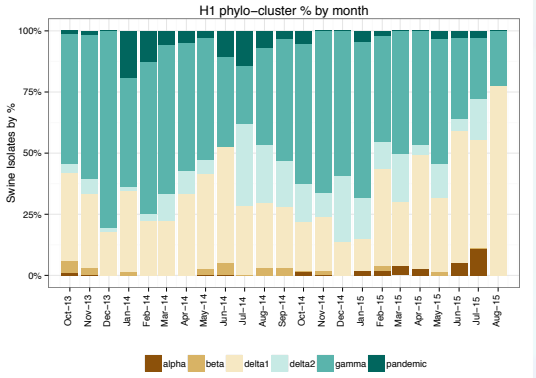


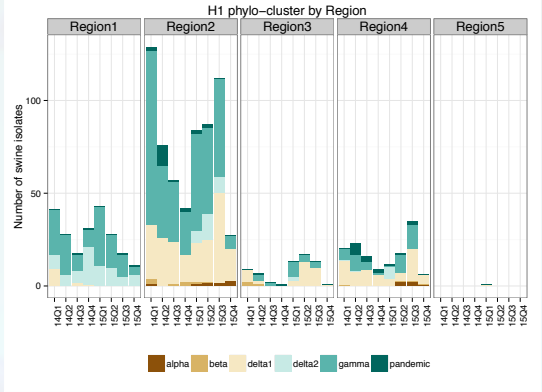
- USDA APHIS Veterinary Services system, active since 2009
- Virus isolates have HA, NA and M sequenced for all, WGS for subset
- Sequences in GenBank and isolates available through USDA NVSL repository
  - A/swine/Arkansas/A01840698/2015
  - http://www.aphis.usda.gov/library/forms/pdf/VS_Form4_9.pdf
  - Email your request to: NVSL_Userfee@aphis.usda.gov

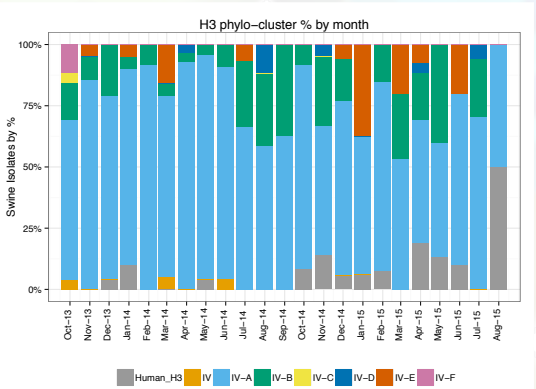
NADC partners to do genetic, antigenic, and phenotypic characterization on viruses of interest.

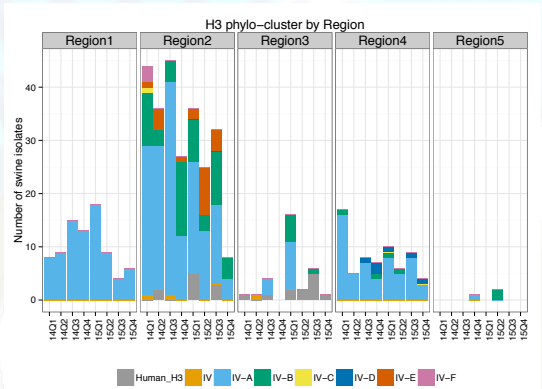
HA Phylogenetic Summaries







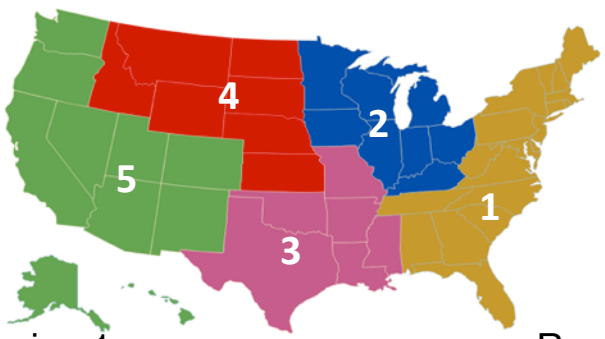




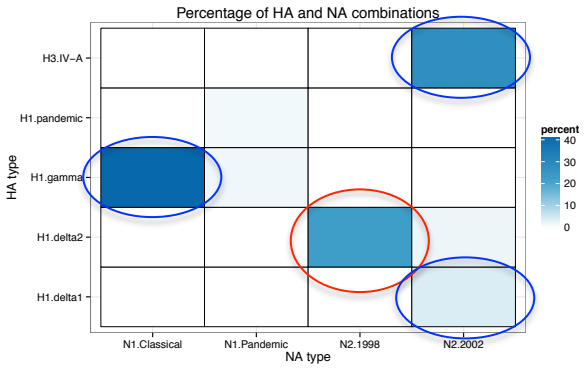
- Gamma H1, delta-1 H1 and CIV-A H3 are most predominant HA nationally
- Extensive diversity overall especially Region 2
- Dominant phylotypes do not seem to replace the minor ones
- Some regional differences

Rasna Walia

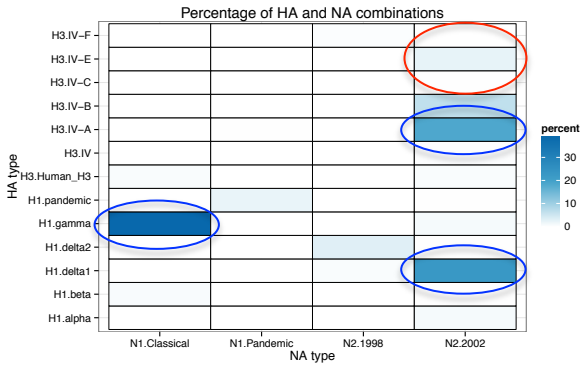
Regional patterns of HA/NA combinations



Region 1

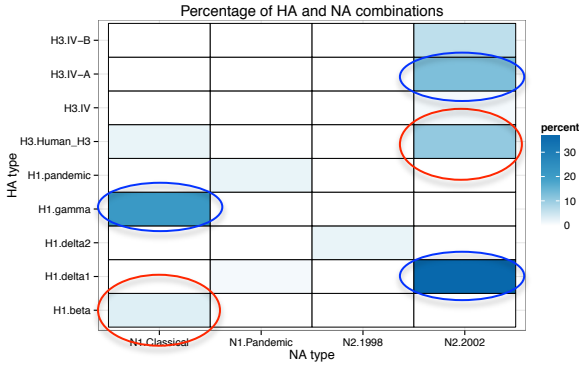


Region 2

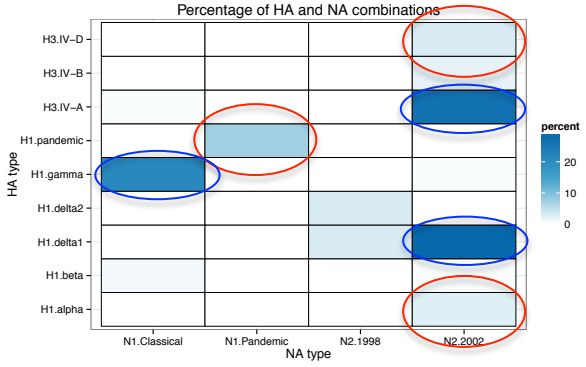


- Common combination across all regions
- Combinations more unique to a region

Region 3



Region 4

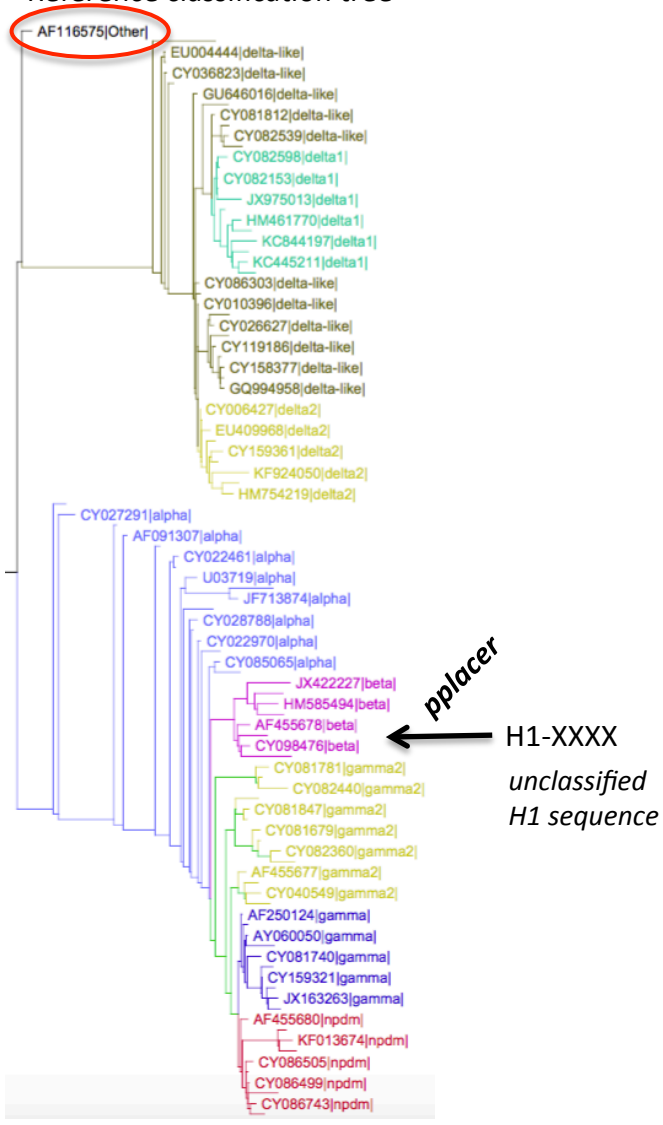


Rasna Walia

Swine H1 clade classification in IRD

C. Macken (IRD) and T. Anderson (USDA)

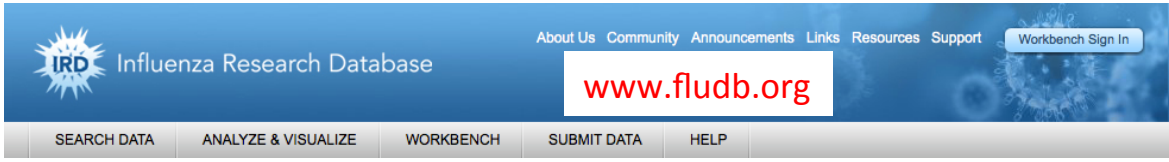
Reference classification tree



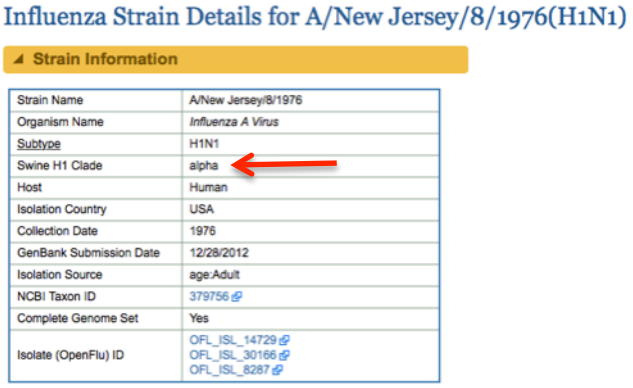
pplacer

H1-XXXX

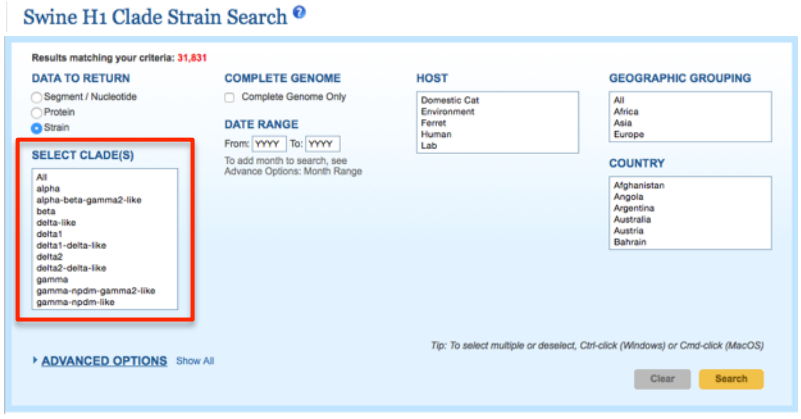
unclassified H1 sequence



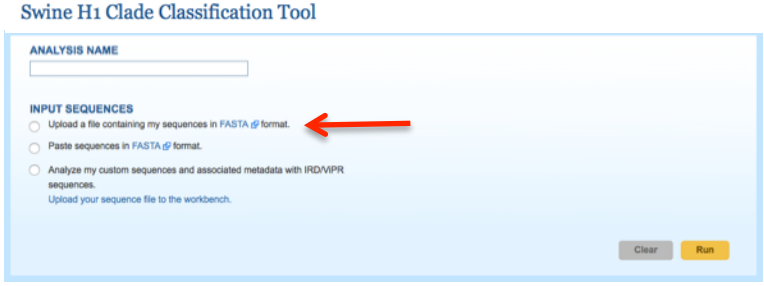
Strain Detail Page
Pre-computed annotations for ***all H1 viruses in all hosts***



Search Data Menu
Clade-specific strain/segment /protein search

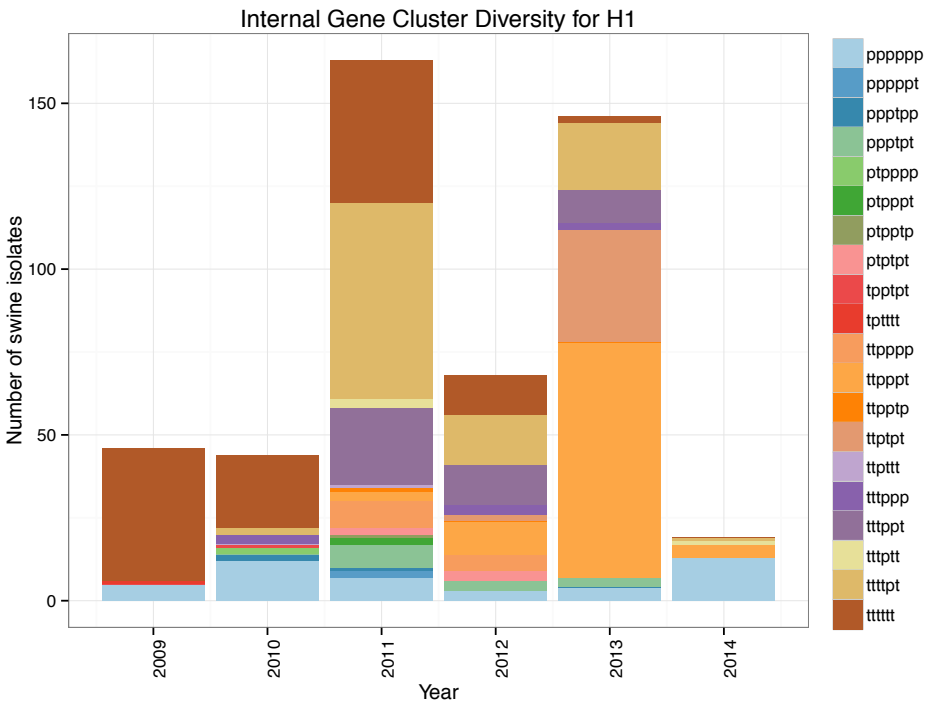


Analyze Tool Menu
Clade assignment of user sequences



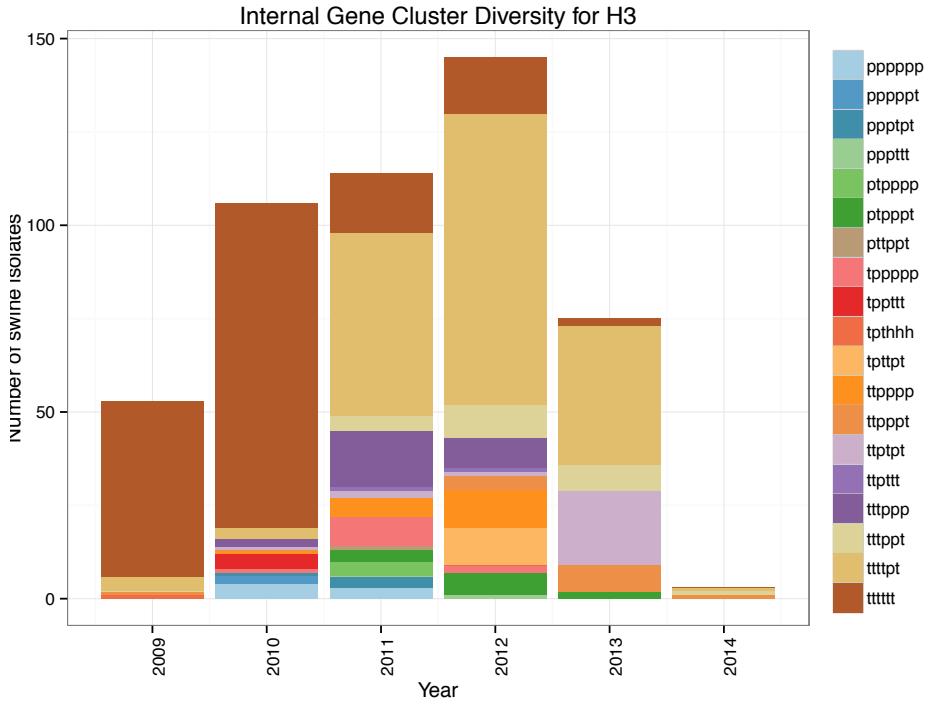
Internal Gene Patterns

H1



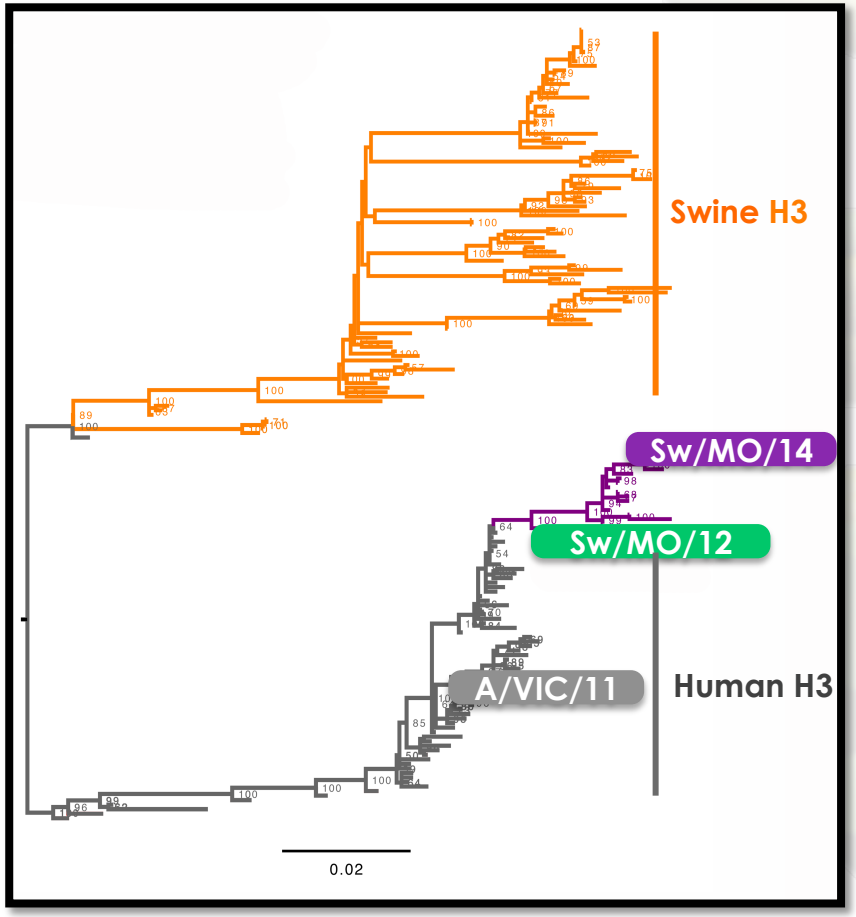
Hylia Gao

H3

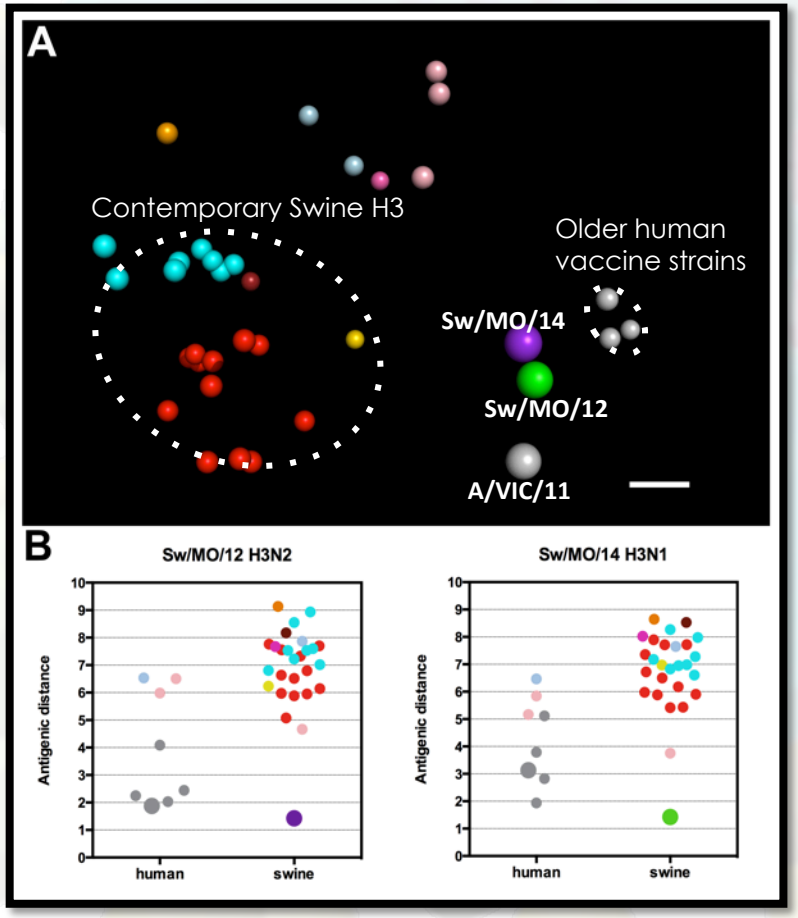


Rasna Walia

Viruses of interest – Human to swine H3



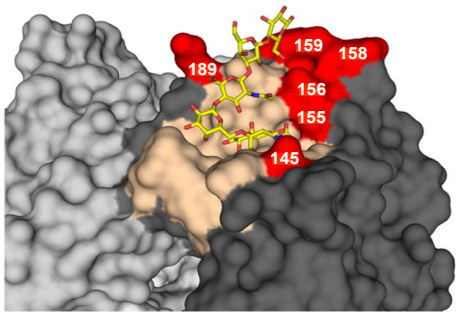
The HA of the human-like H3N2 and H3N1 viruses are genetically similar to human seasonal H3N2 from 2010/2011, documenting a recent spillover event.



The human-like viruses are antigenically distant from contemporary swine H3 viruses and from recent human seasonal H3N2.

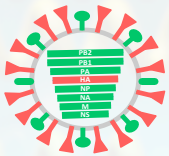
Daniela Rajao

Swine H3 Antigenic Determinants

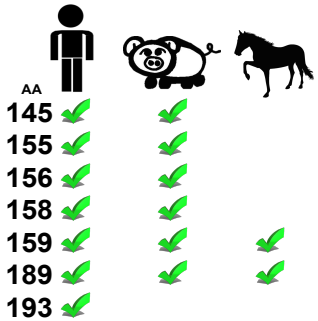


OH/04

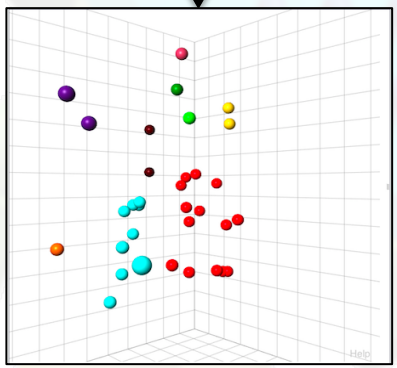
OH/04 mutants (n= 38)



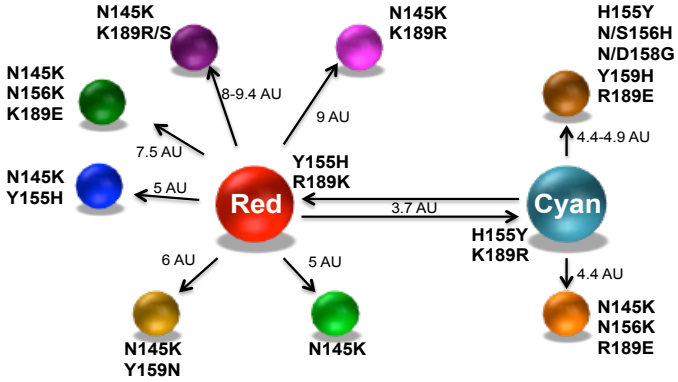
Cross hemagglutination-inhibition (HI) assays performed using a standardized swine sera panel.



HI data used to generate antigenic map.



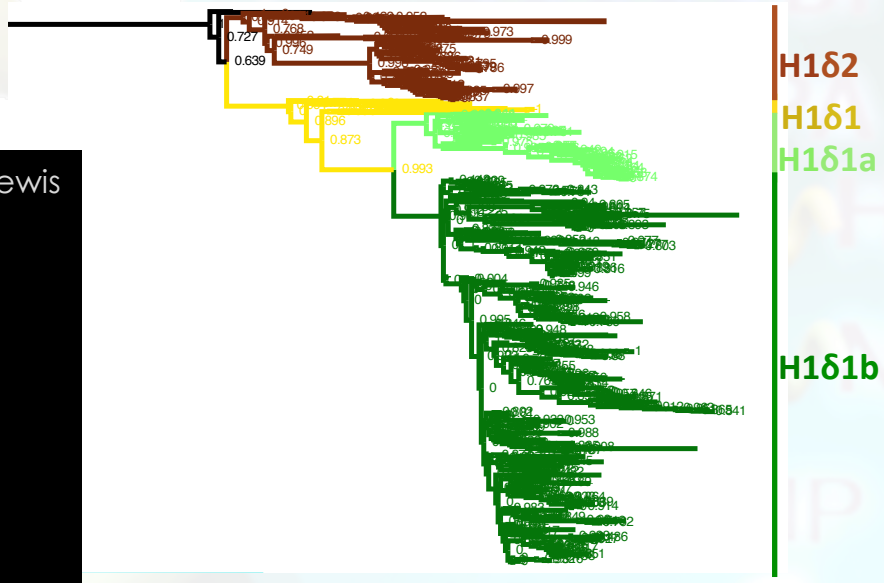
Pathogenesis and transmission studies

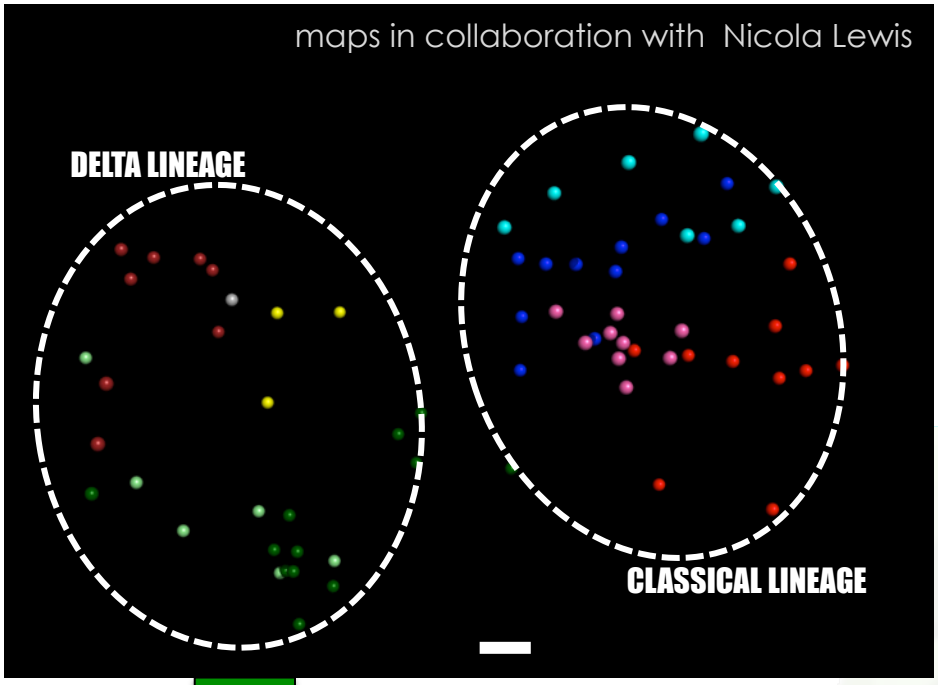


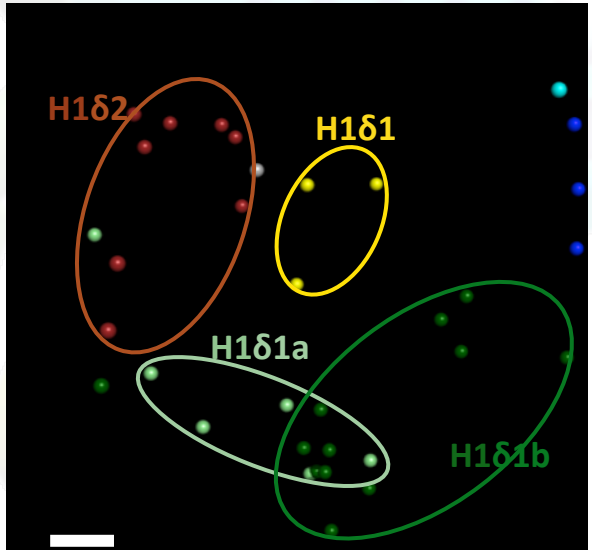
Collaboration with Nicola Lewis, Daniel Perez & Jeff Santos

Eugenio Abente

Antigenic diversity of swine H1 in USA



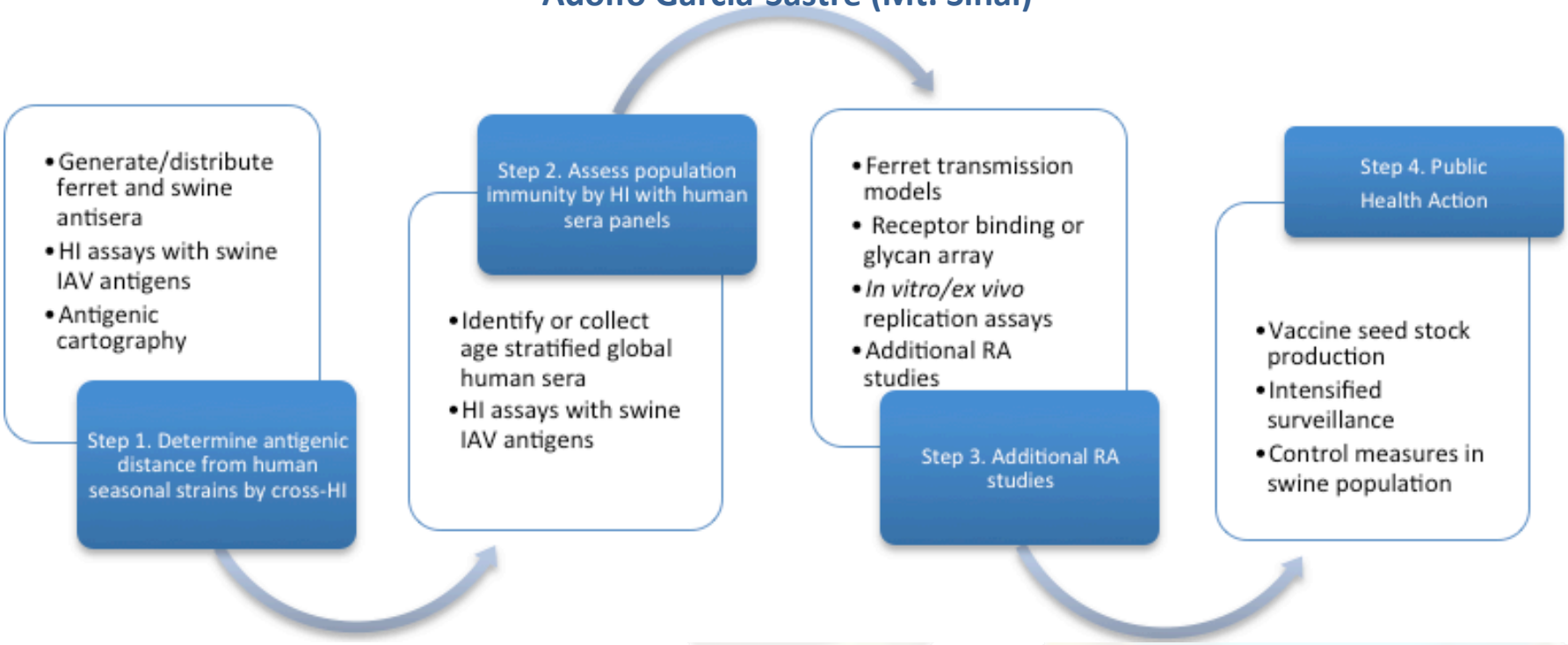




Daniela Rajao

NIH CEIRS: Swine Influenza Pandemic Threats Concept

Amy Vincent (USDA)
Nicola Lewis (University of Cambridge)
Adolfo Garcia-Sastre (Mt. Sinai)



Systematic approach to evaluate risk of swine IAV to the human population based on antigenic distances and human population immunity.

- Swine isolates identified through surveillance and sequencing efforts selected to enter the pipeline.
- Direct comparison of ferret and swine anti-sera.
- Cycle repeated as needed as newly characterized swine IAV are identified.
- An inter-center project: SJCEIRS, JHCEIRS, CRIP, Emory

Human Vaccine Strains for Swine Anti-sera Progress and Future Plans

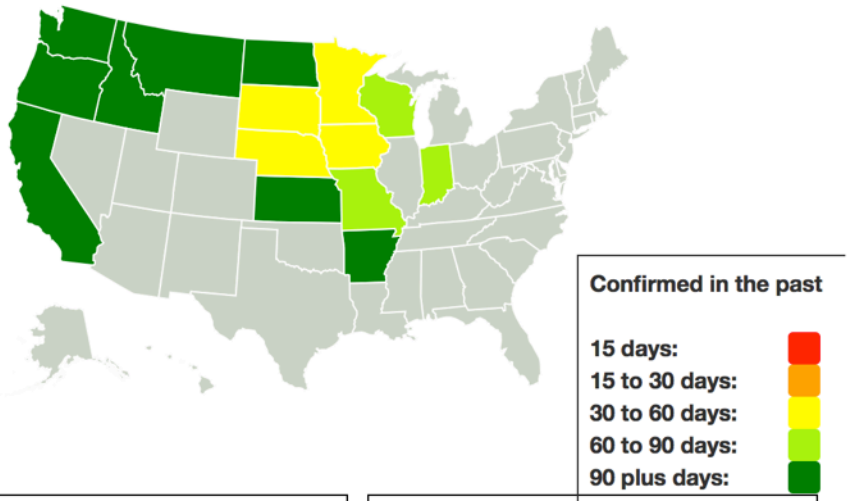
Vaccine Strain	Year	Subtype	Vaccine Season	NADC Sera
A/California/04/09	2009	H1N1	2010-15	Yes
A/Brisbane/59/07	2007	H1N1	2007-10	Yes
A/Solomon Island/3/06	2006	H1N1	2007-08	Yes
A/ Michigan/02/2003	2003	H1N2	N/A	Yes
A/New Caledonia/20/1999	1999	H1N1	2000-07	Yes
A/Victoria/361/2011	2011	H3N2	2012-14	Yes
A/Perth/16/2009	2009	H3N2	2010-12	Yes
A/Brisbane/10/2007	2007	H3N2	2008-10	Yes
A/Wisconsin/67/2005	2005	H3N2	2006-08	Yes
A/Fujian/411/2002	2002	H3N2	2004-05	Yes
A/Moscow/10/99	1999	H3N2	2000-04	Yes
A/Sydney/5/97	1997	H3N2	1998-2000	Yes
A/Wuhan/359/95	1995	H3N2	1996-98	Yes

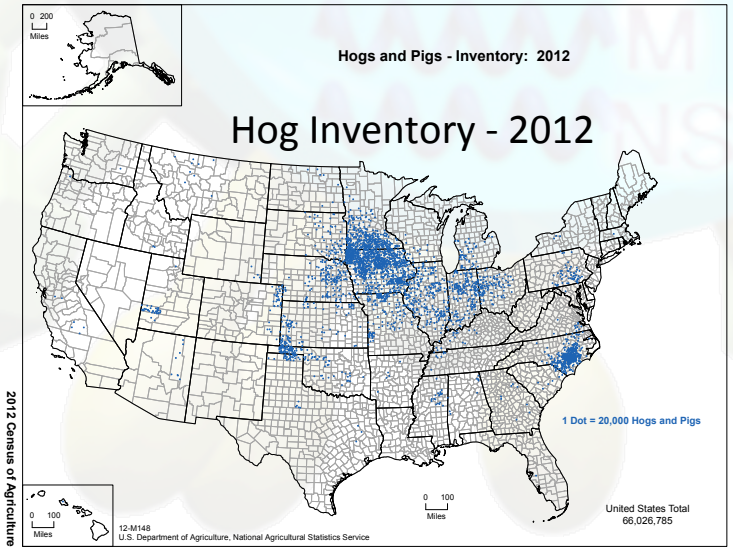
Vaccine Strain	Year	Subtype	Vaccine Season	NADC Sera	Received from St. Jude
A/Singapore/86	1986	H1N1	1987-97	No	✔
A/Brazil/11/78	1978	H1N1	1980-84	No	✔
A/Beijing/262/95	1995	H1N1	1999-2000	No	✔
A/Texas/36/91	1991	H1N1	1995-97	No	✔
A/Taiwan/1/86	1986	H1N1	1987-97	No	✔
A/Chile/1/83	1983	H1N1	1984-86	No	✔
A/USSR/77	1977	H1N1	1978-79	No	✔
A/Switzerland/9715293/13	2013	H3N2	2015-16	No	✔
A/Beijing/32/92	1992	H3N2	1993-94	No	✔
A/Beijing/353/89	1989	H3N2	1991-93	No	✔
A/Sichuan/02/87	1987	H3N2	1988-89	No	✔
A/Shanghai/11/87	1987	H3N2	1989-90	No	✔
A/Leningrad/360/1986	1986	H3N2	1987-88	No	✔
A/Philippines/2/82	1982	H3N2	1983-86	No	✔
A/Bangkok/01/1979	1979	H3N2	1981-83	No	✔
A/Texas/1/77	1977	H3N2	1978-79	No	✔
A/Victoria/3/75	1975	H3N2	1976-78	No	✔
A/Port Chalmers/1/1973	1973	H3N2	1974-76	No	✔

- Measure antigenic distance of Human←→Human and Swine←→Human IAV in the context of swine or ferret antisera.
- Is swine anti-sera a reliable predictor for antigenic distance relevant to humans?

Pathogenesis & transmission studies - HPAI in the swine host

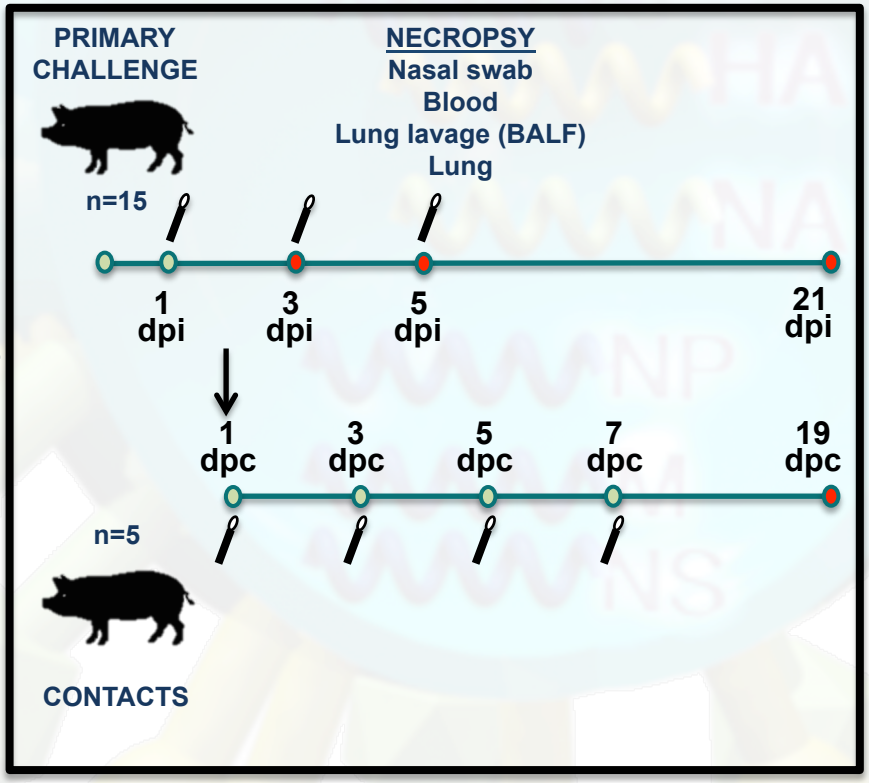
Poultry Cases confirmed by USDA





HPAI H5Nx infection in swine

- Four isolates (3 subtypes) of HPAI:
  - A/turkey/MN/7172-1/15 H5N2
  - A/Northern_pintail/WA/40964/14 H5N2
  - A/gyrfalcon/WA/41088_6/14 H5N8
  - A/American_green_winged_teal/WA/195750/14 H5N1
- Primary pigs were experimentally infected to evaluate infection and shedding properties of the HPAI viruses.
- Contact pigs were placed on 2 dpi to monitor for transmission.
- Subsets of primary pigs were euthanized at 3 and 5 days post infection (dpi) to evaluate replication in the lungs.
- Nasal swabs were collect from primary and contact pigs at multiple time points to evaluate upper respiratory tract replication.
- Blood was collected at necropsy and at 14 and 21 dpi or contact (dpc) from 5 primary and 5 contact pigs to monitor for seroconversion.
- 2 primary pigs each virus were boosted by adjuvanted virus to generate reference anti-sera.



USA H5Nx HPAI
Summary to Date

Virus Strain	NS dpi 1[#]	NS dpi 3[#]	NS dpi 5[#]	Lungs 3*	Lungs 5*	Seroconversion HI	Seroconversion NP ELISA
A/turkey/Minnesota/7172-1/2015 H5N2							
Primary	0	0	0	5 / 3	5 / 3	2	5
Contact	0	0	0	-	-	0	0
A/Northern_pintail/Washington/40964/2014 H5N2							
Primary	0	0	0	5 / 4	5 /4	2	4
Contact	0	0	0	-	-	0	0
A/gyrfalcon/Washington/41088_6/2014 H5N8							
Primary	0	0	1	5 / 5	5 / 3	0	3
Contact	0	0	0	-	-	0	0
A/American_green_winged_teal/Washington/195750/2014 H5N1							
Primary	0	0	0	5 / 4	4 / 3	0	5
Contact	0	0	0	-	-	0	0

[#]Nasal Swabs and Lungs were screened by RT-PCR. Positives were attempted for virus isolation in eggs.

*Number RT-PCR positive / Number Virus Isolation positive.

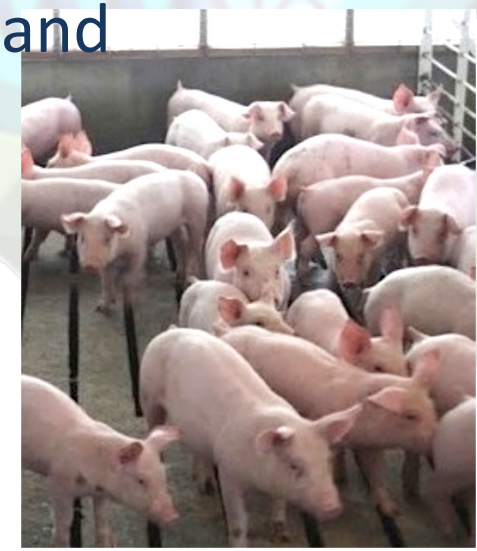
- All lungs were negative for IAV by immunohistochemistry by the standard D-lab anti-NP antibody.

HPAI Take Home Points

- Most avian H5 and H7 viruses previously tested in swine failed to sustain efficient replication and transmission, including the U.S. H5Nx HPAI.
- Low level infection can allow reassortment, so must be vigilant if there are new poultry outbreaks during the 2015-16 waterfowl migratory season. Pay attention to the turkey-swine influenza niche.
- Risk ≠ 0, but seems to be unlikely or self limiting without multiple mutations and reassortment events.
- The new canine H3N2 also appears to be low in risk for transmission to swine.

Summary of recent activities

- NADC is interagency partners with the USDA IAV-S APHIS surveillance system and with NIH-CEIRS.
- Phylogenetic studies
  - Address questions about virus evolution in swine
  - Feeds into our antigenic and phenotypic studies
- Antigenic characterization
  - X-CEIRS virus-host swine pipeline
  - Feeds into our swine vaccine research objectives
- Phenotypic characterization – Pathogenesis and transmission in the natural host
  - Human to swine H3 introductions
  - U.S. H5Nx HPAI isolates
  - U.S. canine H3N2 isolate



Acknowledgements

NADC

- Daniela Rajao
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- Kelly Lager
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- Justin Miller
- Keiko Sampson
- Aaron Hebeison
- Brad Pithan

- Nicola Lewis
- Daniel Perez
- Jefferson Santos
- Alicia Janas-Martindale
- Sabrina Swenson
- Ellen Kasari
- Mia Torchetti
- David Suarez
- Mary Pantin-Jackwood
- Tonia McNunn
- Rebecca Cox
- Martha Nelson
- Marie Culhane
- Phil Gauger
- Catherine Macken
- Richard Scheuermann

CRIP

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Centers of Excellence for Influenza Research and Surveillance

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