Pandemic H1N1 influenza A virus in pigs

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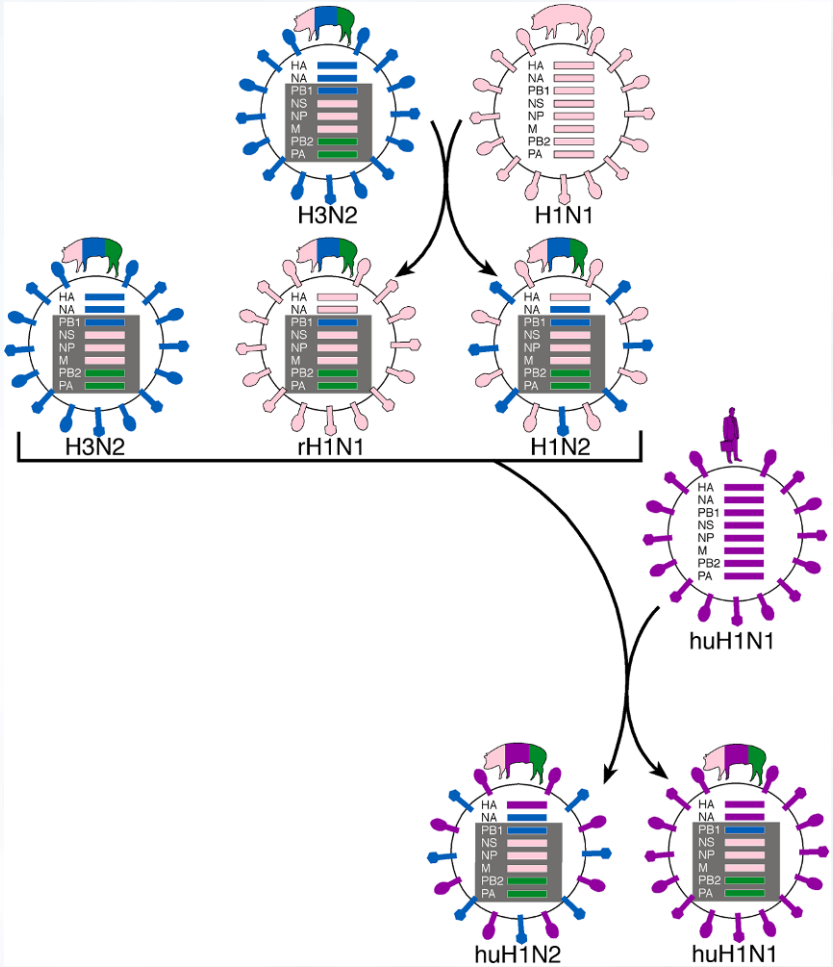
Swine Influenza Research - BP

- Virus pathogenesis & transmission in pigs
  - Novel or emerging SIV – huH1; H2N3
  - Cross-species transmission – HPAI H5N1; LPAI H5 & H7
- Molecular and antigenic characterization
  - Pyrosequencing for whole genome sequencing
  - HI and antigenic cartography
- Vaccine studies in pigs
  - RG attenuated virus - MLV
  - Vectored subunit - adenovirus
  - Whole virus inactivated
- Diagnostic test development/application
  - ELISA – IDEXX multispecies AI
  - Rapid antigen tests
- Human/Animal interface project with CDC and USDA-APHIS
  - OH/07 county fair event
  - H2N3

Circulating SIV In North America

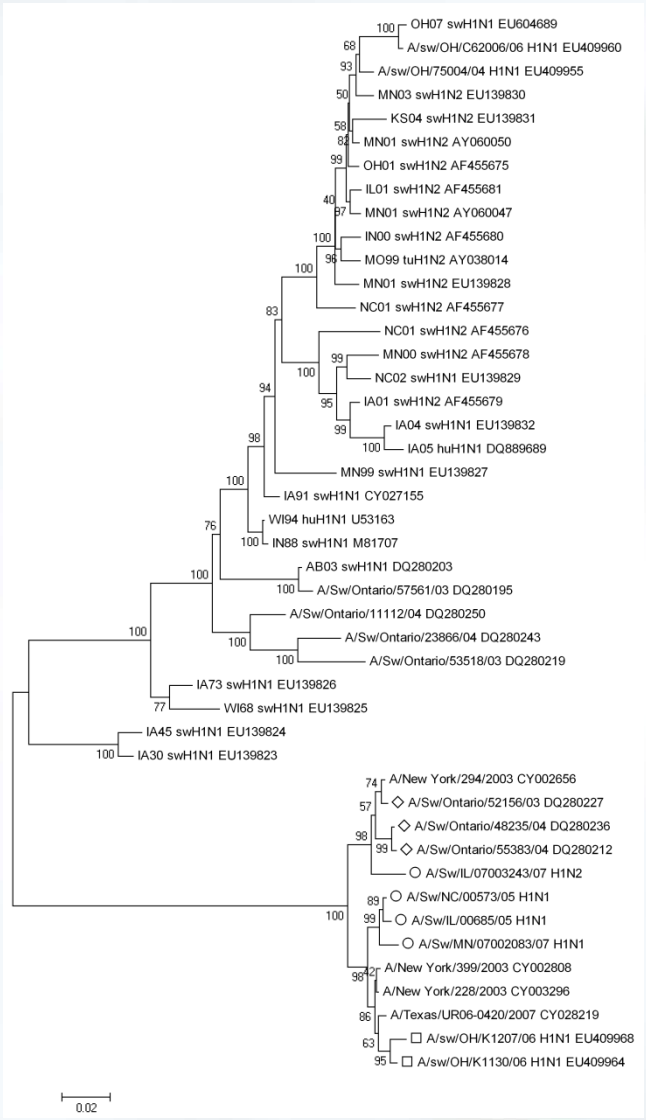


- H3N2
- cH1N1
- rH1N1
- H1N2
- huH1N1
- huH1N2

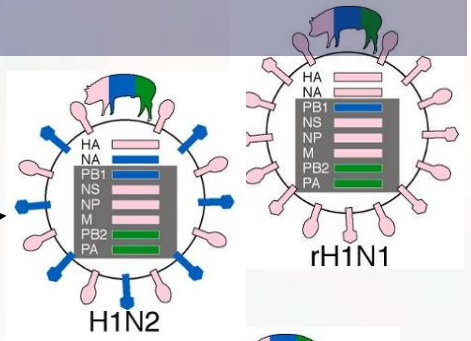
- **Triple reassortant internal gene (TRIG) cassette**

Swine H1 Hemagglutinin Genes

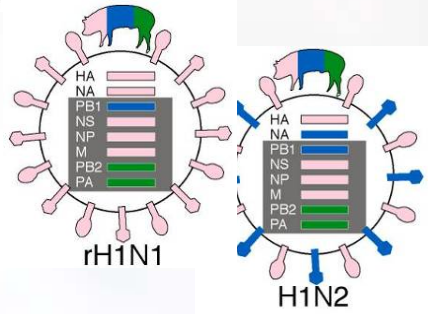
HA



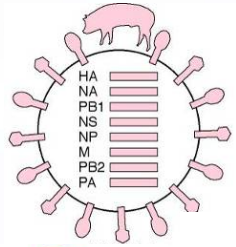
swH1γ



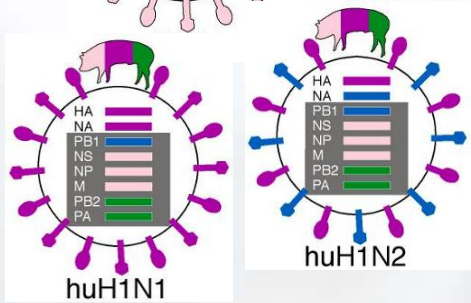
swH1β



swH1α



swH1δ



2009 Pandemic H1N1 Research – AP

- Virus pathogenesis & transmission in pigs
  - Virus tissue distribution in pigs (H1N1)v
- Molecular and antigenic characterization
  - HI and antigenic cartography
- Diagnostic test development/application
  - RT-PCR
  - ELISA
  - Rapid antigen tests: BinaxNow, FluDetect
- Vaccine studies in pigs
  - Whole virus inactivated
  - RG attenuated virus MLV

NADC Pandemic H1N1 studies

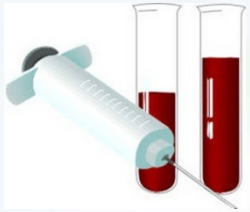
- Pilot pathogenesis study on 4 pigs sampled at 5 dpi
  - A/CA/04/2009 (H1N1)v
- Lung lesions typical of influenza virus
- All non-respiratory tract tissues were negative by virus isolation
  - No evidence for microscopic lesions in:
    - Liver, kidney, spleen, lymph node, tonsil or skeletal muscle
  - No evidence for antigen by IHC in:
    - Liver, kidney, spleen, lymph node, tonsil or skeletal muscle

2009 H1N1 – Transmission

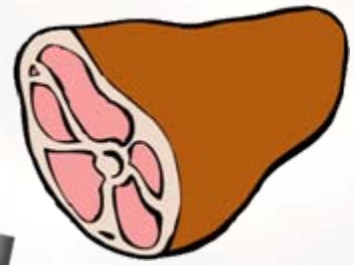
- Larger pathogenesis and transmission study
  - Two pandemic virus isolates tested:
    - A/CA/04/2009 (H1N1)v
    - A/Mexico/4108/2009 (H1N1)v
  - Pigs sampled at 3, 5 & 7 dpi
  - All non-respiratory tract tissues were negative by virus isolation
  - Virus transmitted to direct and indirect contacts
- Differences between A/CA/04/09 and A/MX/4108/09
  - Nasal shedding and kinetics of transmission
  - Difference as antigens in HI

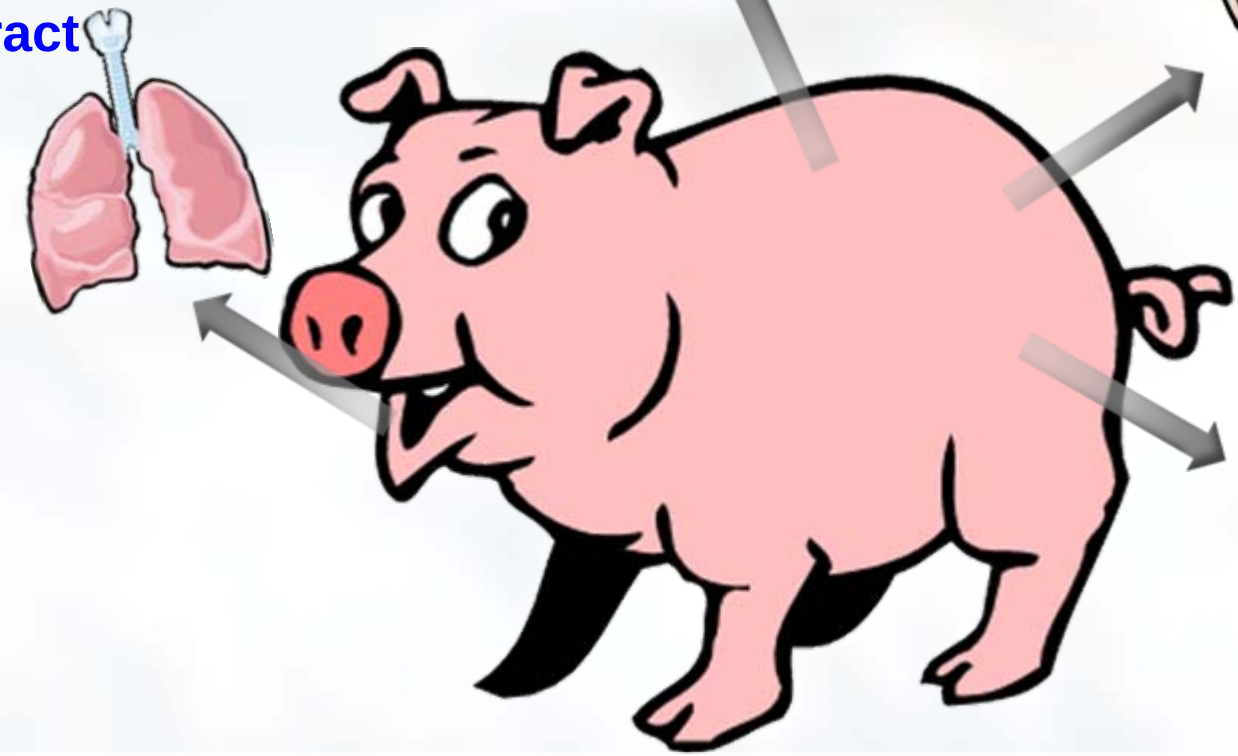
(+)
Respiratory tract

(-)
Blood



(-)
Ham





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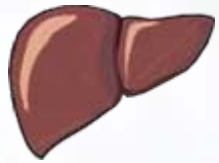
Internal organs:
Liver
Spleen
Kidney
Intestinal Contents



Take Home Message:

H1N1 Flu virus found **only in respiratory tract.**
All other tissues were **NEGATIVE.**
Sick pigs are not sold for slaughter.

USDA ARS NADC National Animal Disease Center

Serologic cross-reactivity – Endemic SIV panel

H1α Phylogenetic cluster (3 viruses)

Antiserum specificity	Homologous HI titer	CA/09 HI titer	Fold-Reduction CA/09	MX/09	Fold-Reduction MX/09
MN/37866/99	1280	20	64	160	8
	2560	80	32	320	8
MN/02053/08	320	<10	320	20	16
	320	<10	320	<10	320
MN/02093/08	320	<10	320	<10	320
	320	<10	320	<10	320

H1β Phylogenetic cluster (5 viruses)

Antiserum	Homologous HI titer	CA/09 HI titer	Fold-Reduction CA/09	MX/09 HI titer	Fold-Reduction MX/09
NC/36883/02	640	40	16	160	4
	640	20	32	80	8
IA/00239/04	1280	20	64	80	16
	1280	20	64	40	32
KY/02086/08	80	<10	80	<10	80
	640	<10	640	20	32
IA/02096/08	160	<10	160	<10	160
	80	<10	80	<10	80
NE/02013/08	640	<10	640	<10	640
	160	<10	160	<10	160
NC/03084/08	640	<10	640	<10	640
	320	<10	320	<10	320

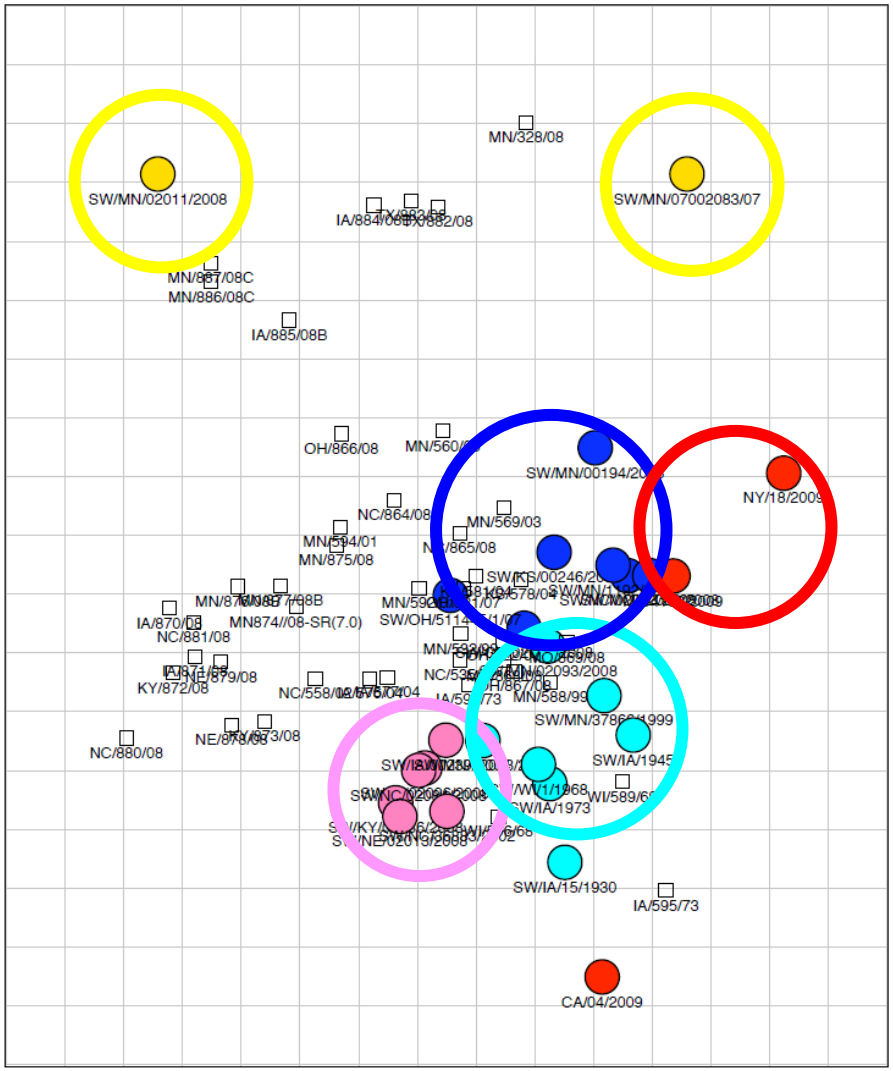
H1γ Phylogenetic cluster (8 viruses)

Antiserum	Homologous HI titer	CA/09 HI titer	Fold-Reduction CA/09	MX/09 HI titer	Fold-Reduction MX/09
OH/511445/07	640	20	32	160	4
	2560	80	32	640	4
MO/02060/08	1280	40	32	160	8
	640	80	8	320	2
OH/02026/08	640	40	8	80	8
	160	<10	160	10	16
NC/02023/08	320	<10	320	40	8
	320	<10	320	80	4
KS/00246/04	1280	<10	1280	160	8
	1280	<10	1280	160	8
MN/00194/03	1280	<10	1280	160	8
	320	<10	320	320	1
MN/1192/01	320	<10	320	160	2
	80	<10	80	20	4

H1δ phylogenetic cluster (3 viruses)

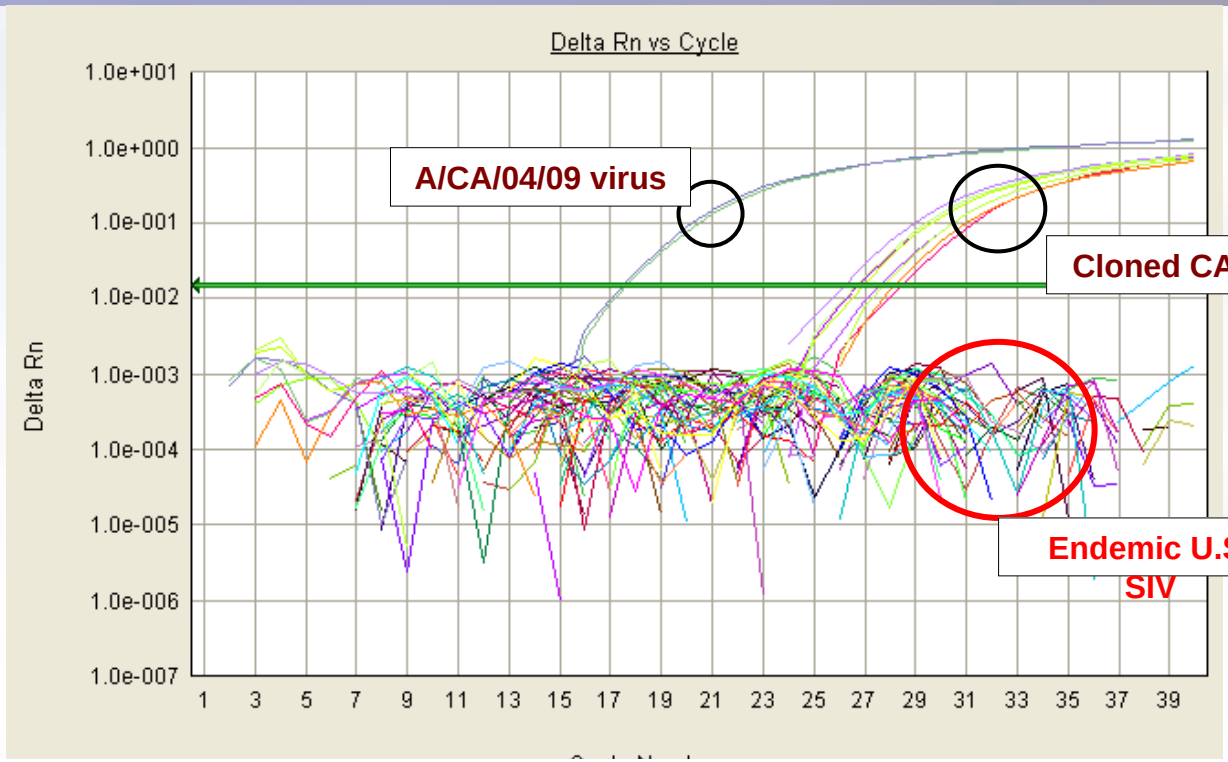
Antiserum	Homologous HI titer	CA/09 HI titer	Fold-Reduction CA/09	MX/09 HI titer	Fold-Reduction MX/09
TX/01976/08	320	<10	320	<10	320
	160	<10	160	<10	160
IA/02039/08	320	<10	320	<10	320
	160	<10	160	<10	160
MN/02011/08	2560	<10	2560	<10	2560
	640	<10	640	<10	640

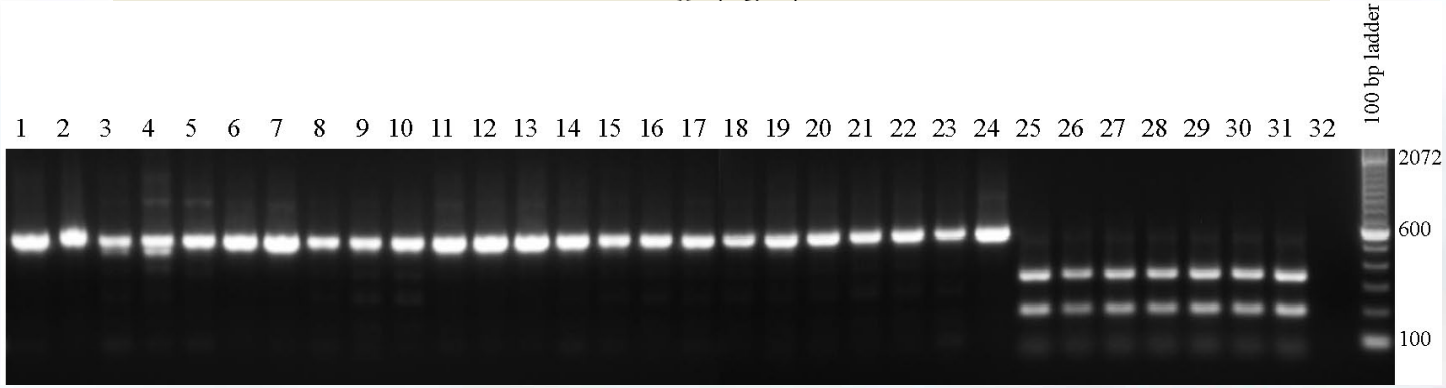
Antigenic Cartography – pandemic H1N1



- Adding the pandemic H1 against swine sera
- Cambridge University – Nicola Lewis

Differential Matrix RT-PCR Assays





Additional Research Activities

- Vaccine studies underway
  - Inactivated vaccine challenge
  - MLV study
  - Natural exposure
- Interaction with CVB and NVSL
  - Generation of immune-sera
  - H1N1 virus stocks – CVB
  - Differential matrix assays – NVSL
  - Surveillance/Research partnership – NVSL
- Research Collaboration
  - CDC: SIV receptor binding
  - Cambridge: Antigenic cartography
  - ISU: Differential ELISA
  - International SIV projects: Russia, Brazil, Vietnam (proposed)

Why is the information on SIV limited?

It’s not sexy work!