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Rational Development of Influenza Vaccines: NDV-based influenza vaccines for poultry and livestock

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KANSAS STATE
UNIVERSITY



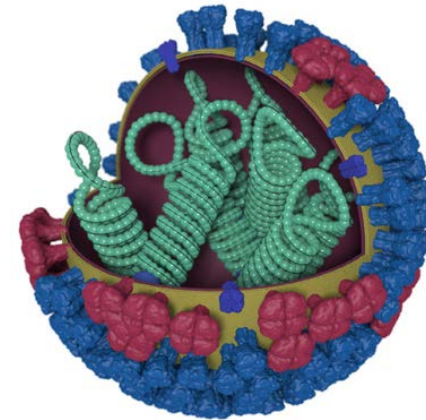
Presentation outline

- **Influenza Virus and Evolution of Influenza**
- **Universal Influenza Vaccine Concept**
- **Newcastle Disease Virus (NDV) as Vaccine Vector for Influenza**
- **Development and testing of NDV-based vaccines against different serotypes of Avian Influenza**
- **Summary/Conclusions**

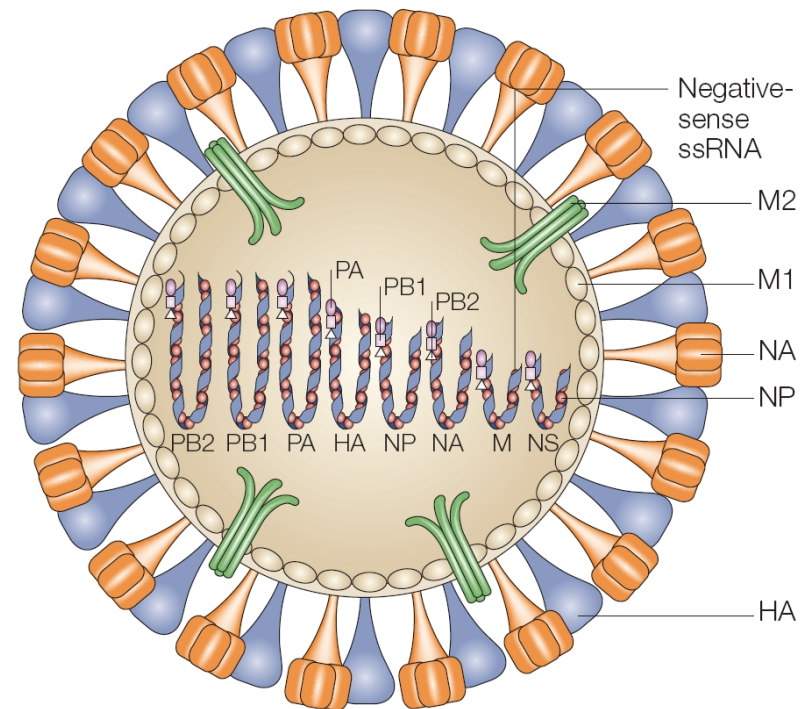


Influenza Viruses

- ✓ RNA viruses
- ✓ Family : *Orthomyxoviridae*
 - Influenza A: birds, swine, humans, other species
 - Influenza B: humans, seal, ferret, swine
 - Influenza C: humans
 - Influenza D: cattle
- ✓ 80-120 nm
- ✓ 11 genes on 8 segments of RNA
- ✓ Proteins: HA, NA, NP, M1, M2, NS1, NS2, PA, PB1, PB1-F2 and PB2



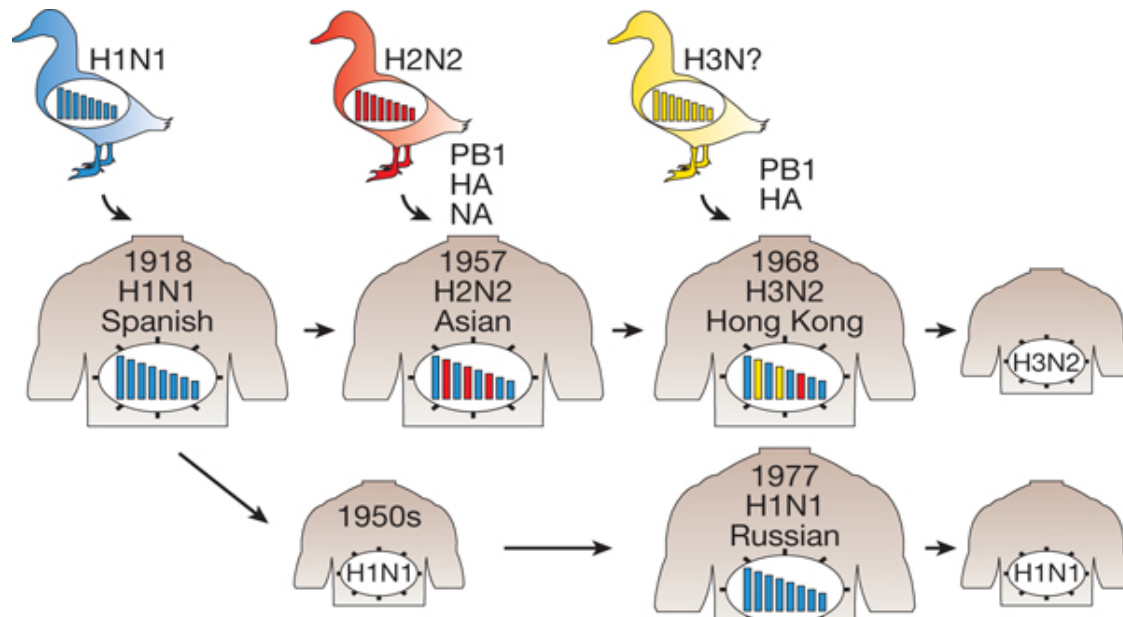
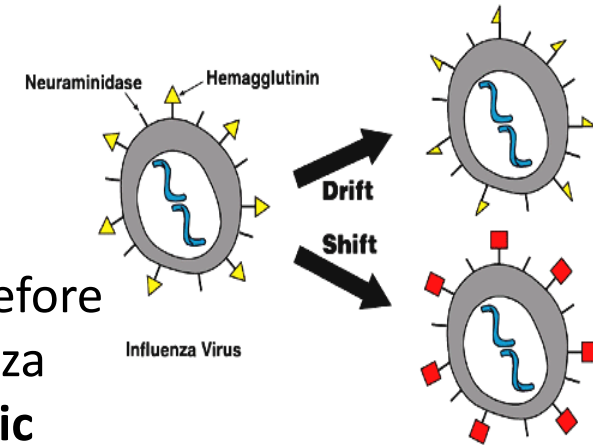
<http://www.cdc.gov/flu/images/virus>





Evolution of Influenza Viruses

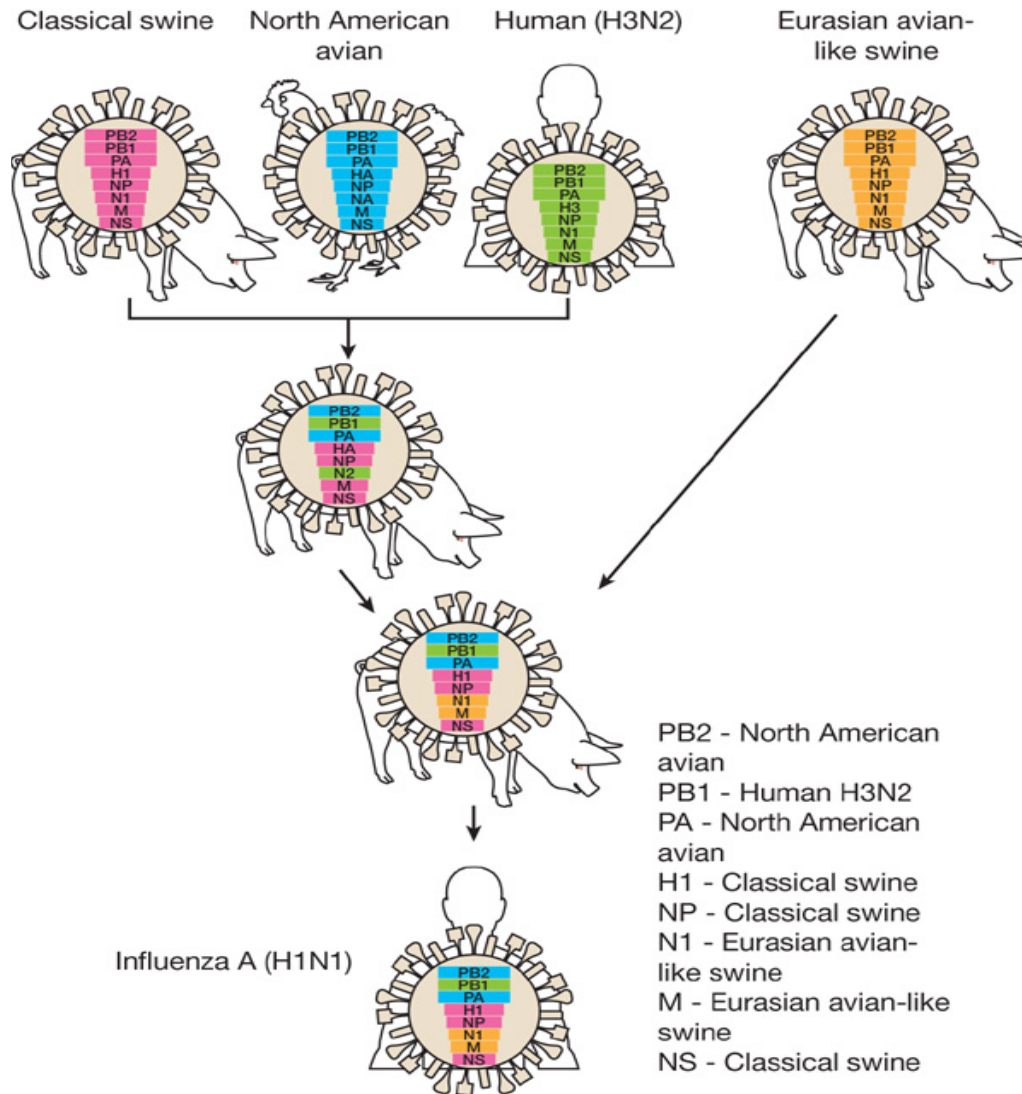
- **Antigenic Drift** : small changes in the HA and NA surface antigens of the virus; often causing an **epidemic/epizootic**
- **Antigenic Shift (reassortment)**: influenza viruses reassort, i.e. they acquire completely new RNA segments/antigens; new antigens have not seen before by humans, and then the novel reassortant influenza virus will spread uncontrollably, causing a **pandemic**



Emergence of pandemic influenza viruses



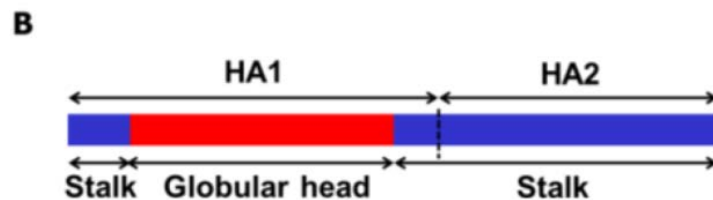
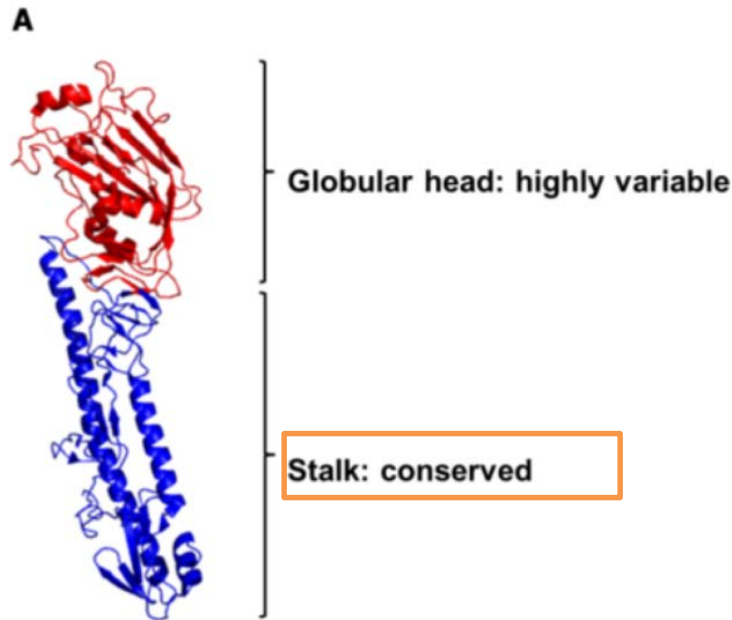
Evolution of Swine Influenza Viruses in the U.S.



- In the late 1990s, **triple reassortant H3N2 and H1N2** swine viruses were isolated from U.S. swine: human H3N2, North American avian, and classical swine viruses -> circulated in North American pig populations
- A triple reassortant swine virus reassorted with a Eurasian avian-like swine virus, that are now circulating in humans -> **pandemic H1N1**



Concept of Universal Influenza Vaccines: The HA Stalk Domain



The function of the HA protein:

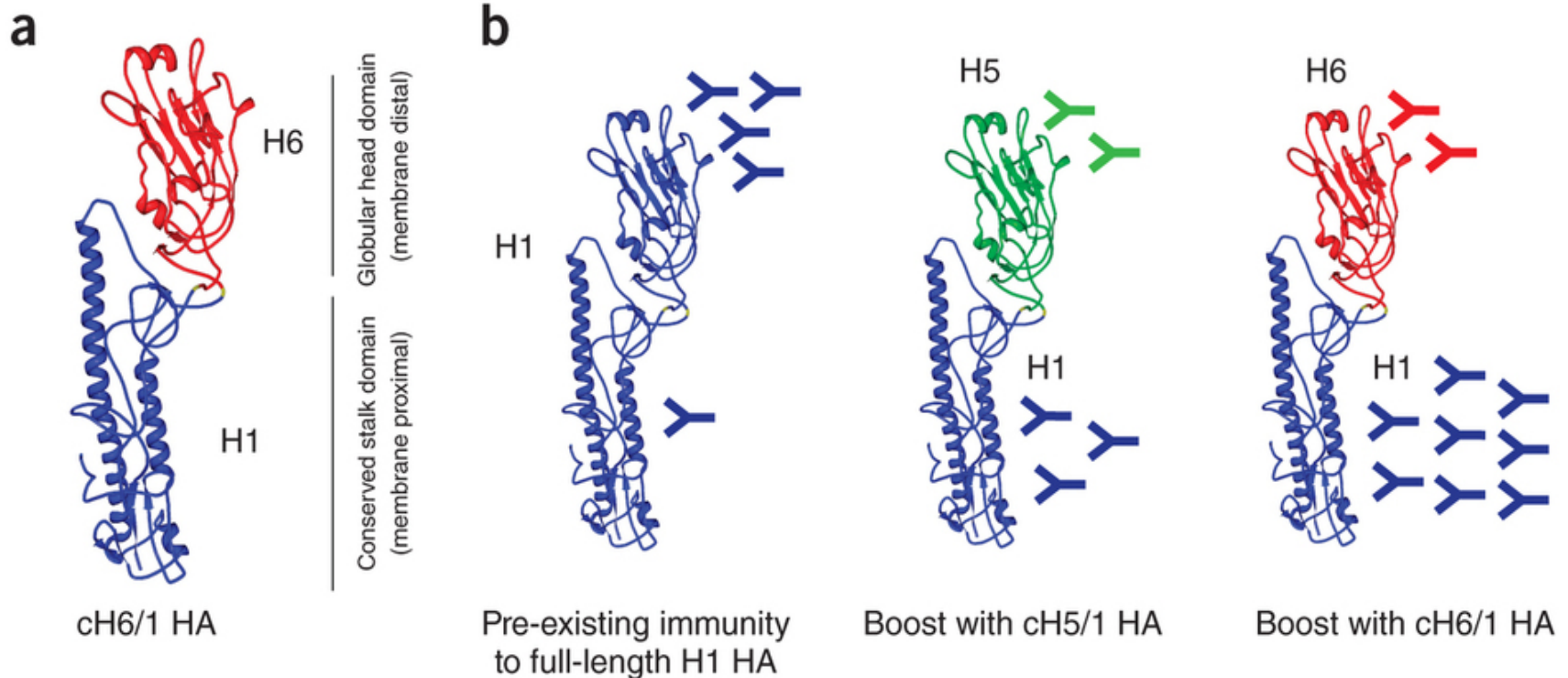
- ✓ A membrane-distal globular head domain: mediates host-cell receptor binding
 - Immunodominant
 - Highly variable
- ✓ A membrane-proximal stalk domain: directs envelope fusion with the host cell
 - Immuno-subdominant
 - Highly Conserved



The Universal Influenza Vaccine Concept

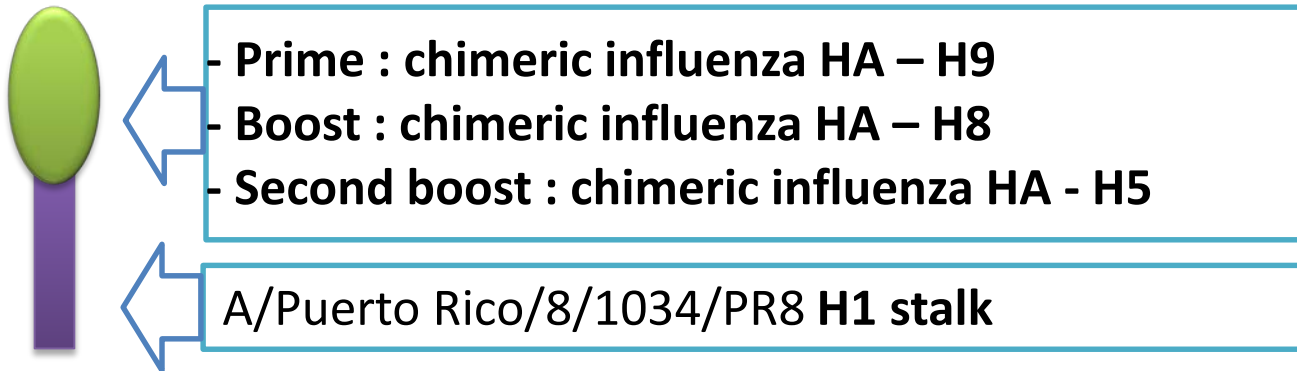
- ✓ Concept is based on shifting the protective humoral immune response towards the antigenically conserved **HA stalk region**
- ✓ Protects against homologous, drifted and shifted influenza virus strains

Example:





Universal Influenza Vaccine Study in Pigs



1. Prime vaccination with Live influenza B virus expressing cH9/H1
2. First boost with inactivated influenza virus construct expressing cH8/H1
3. Second boost with an inactivated influenza virus expressing cH5/H1
4. Booster vaccinations and challenge (pH1N1 CA9) were done 4 weeks apart
5. Piglets enrolled in the study had maternal antibodies to pH1N1



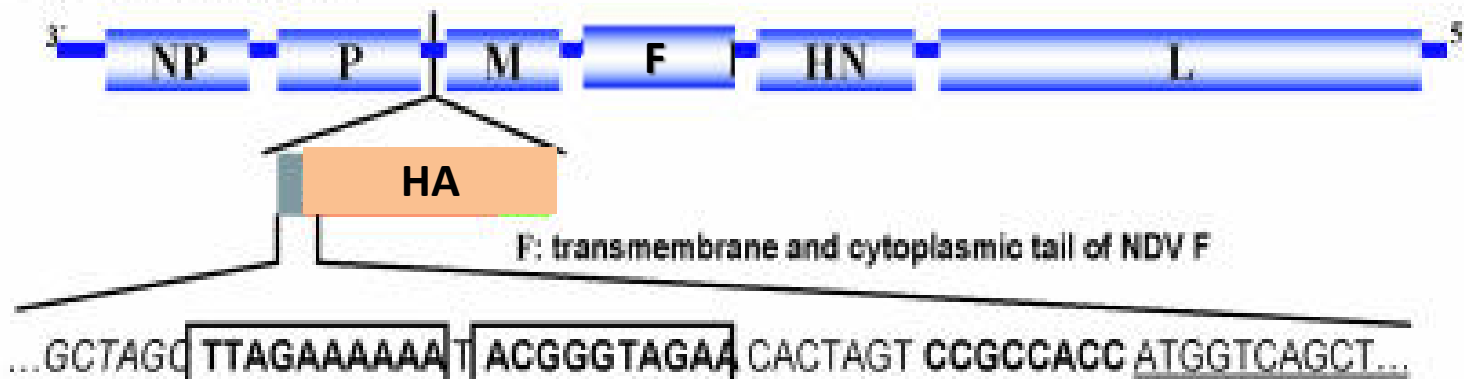
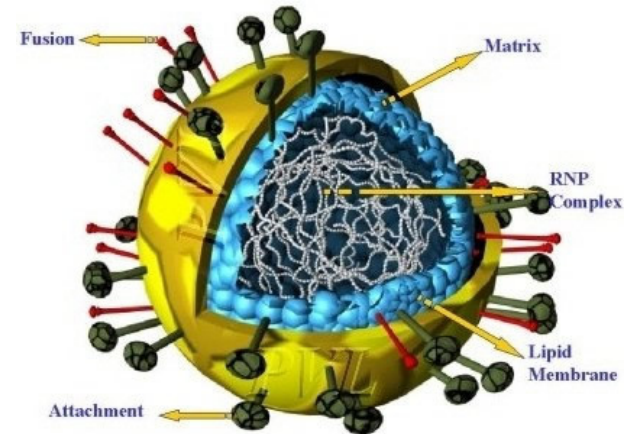
Summary and Conclusions

- Virus titer in nasal swabs of universal vaccine group was decreased on 5 dpc (10^2 vs 10^5 in controls)
- Virus was not detected in BALF of universal vaccine group (Vs 10^3 in controls)
- Universal vaccine group showed significant reduction in macroscopic lung lesions (1% vs 5% in controls)



NDV-based Vaccine Candidates

- Family *Paramyxoviridae*
- Single-stranded, negative sense RNA genome
- Genome is approximately 15 kb in length.
- Deadly disease for poultry – use of LaSota vaccine strain
- Reverse genetics available (based on attenuated NDV LaSota strain)
- Recombinant NDV production using reverse genetics is well established (**4 weeks!!**)





NDV-based Vaccine Vector

Advantages of NDV-based vaccines:

- Are able to differentiate Infected from Vaccinated Animals (DIVA).
- System usable to rapidly develop influenza vaccines based on circulating strains --- within 4-6 weeks
- Prevents both NDV and influenza infections --- one stone-two birds approach.
- NDV vector has been licensed by Avimex Inc. for veterinary purposes, and is being currently used for the manufacturing of H5 influenza vaccines for poultry in Mexico.



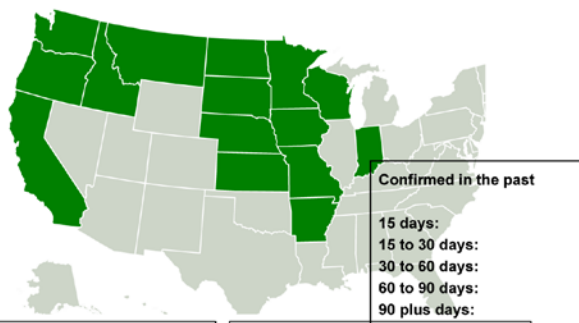
Highly Pathogenic Avian Influenza Viruses (subtype H5; clade 2.3.4.4) outbreaks in the U.S.

USDA United States Department of Agriculture
Animal and Plant Health Inspection Service

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ALL Findings

Update on Avian Influenza Findings
Poultry Findings Confirmed by USDA's National Veterinary Services
Laboratories



219
Detections Reported

48,082,293
Birds Affected

12/19/14
First Detection Reported

6/17/15
Last Detection Reported

View detailed list of
detections

Download list of
detections

Full List of Detections by State

State	Flyway	Confirmed Detections	Last Detection Reported	Total Birds
Arkansas	Mississippi	1	March 11, 2015	40,020

➤ **3/219** outbreaks caused by the Eurasian H5N8 (12/19/2014; 1/23/2015; 2/23/2015)

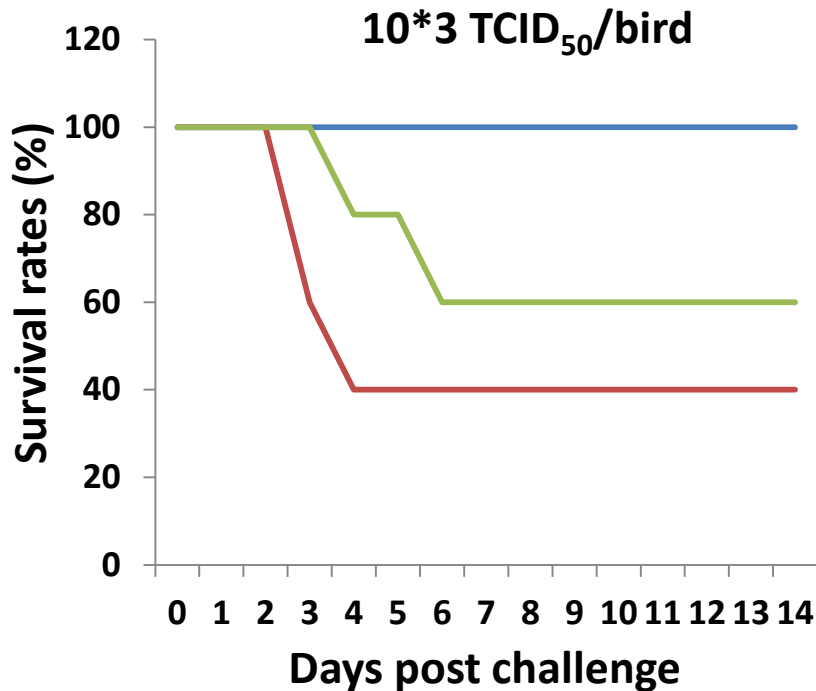
➤ **216/219** outbreaks caused by the novel reassortant H5N2

- ✓ H5N8: an Eurasian virus
- ✓ **H5N2**: reassortant virus between Eurasian H5N8 and North American LPAIVs (**PB1, NP and NA**).
- ✓ H5N1: reassortant virus between Eurasian H5N8 and North American LPAIVs (**PB1, PA, NA and NS**).

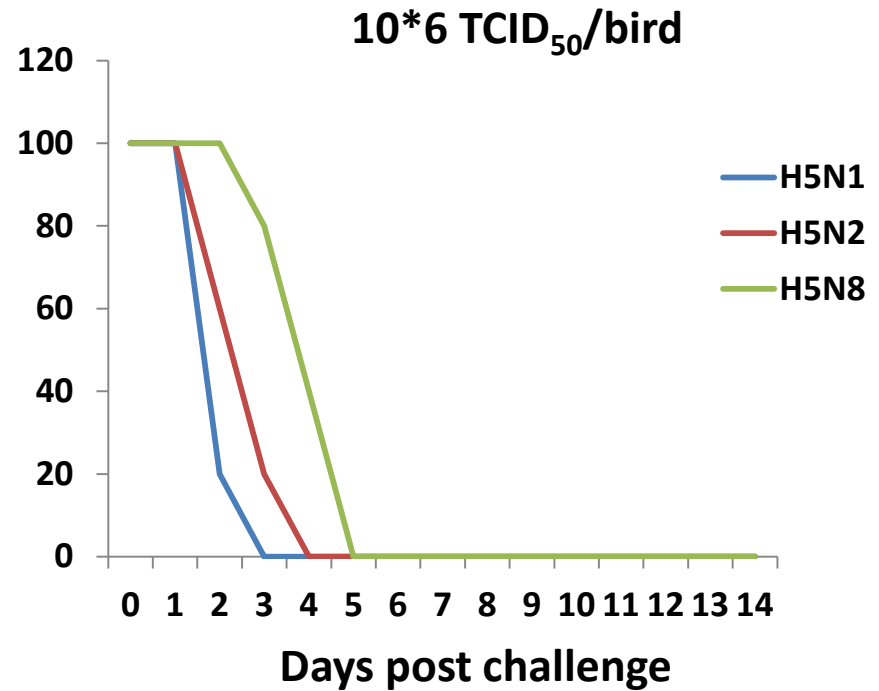


Pathogenicity of highly pathogenic H5Nx viruses in 2-week-old chickens

1. A/American green-winged teal/WA/195750/2014 (H5N1)
2. A/turkey/MN/9845-4/2015 (H5N2)
3. A/gyrfalcon/WA/41088/2014 (H5N8)



H5N2 > H5N8 > H5N1



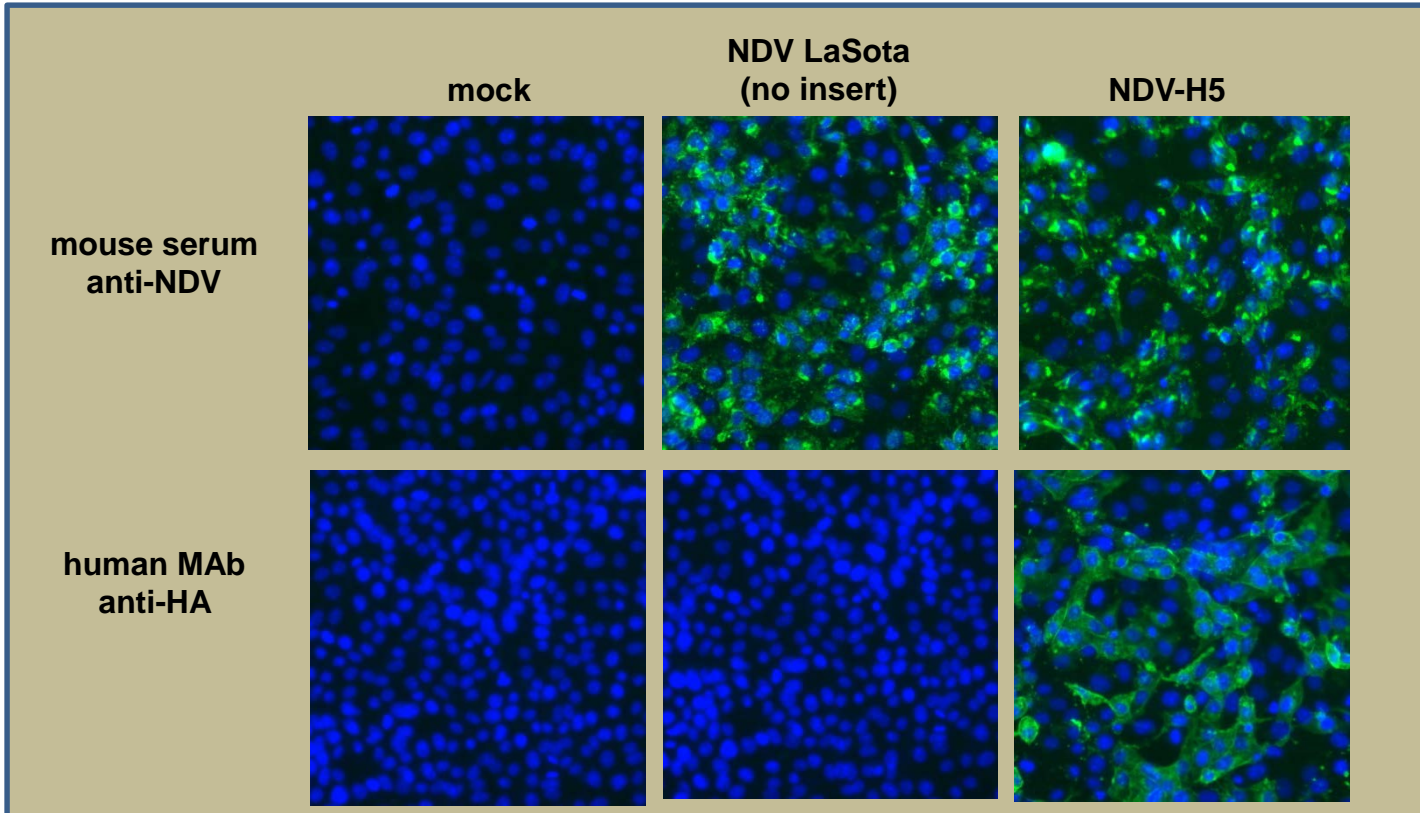
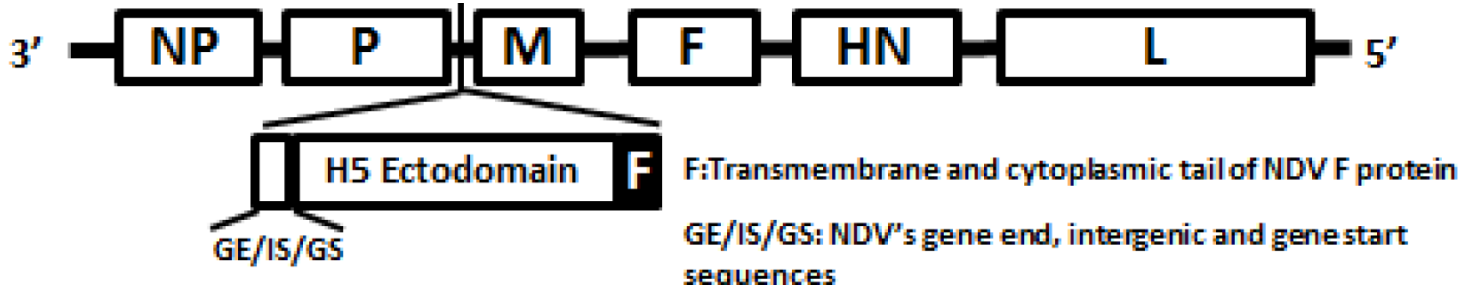
H5N1 > H5N2 > H5N8

H5N2: A/turkey/Minnesota/9845-4/2015 (10⁶ TCID₅₀)



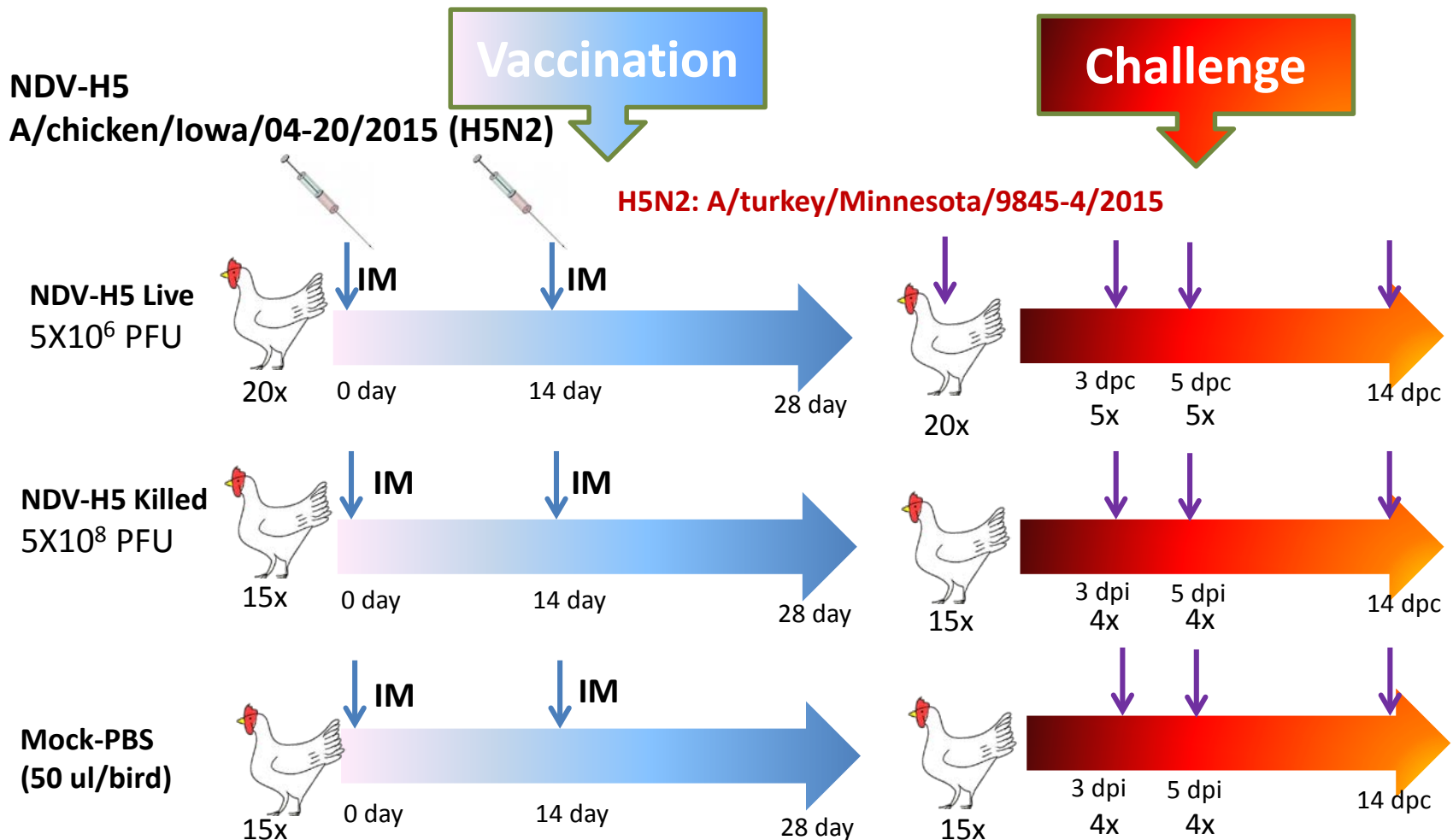
Generation and characterization of recombinant NDV virus expressing HA of highly pathogenic H5N2 virus

A/chicken/Iowa/04-20/2015 (H5N2)





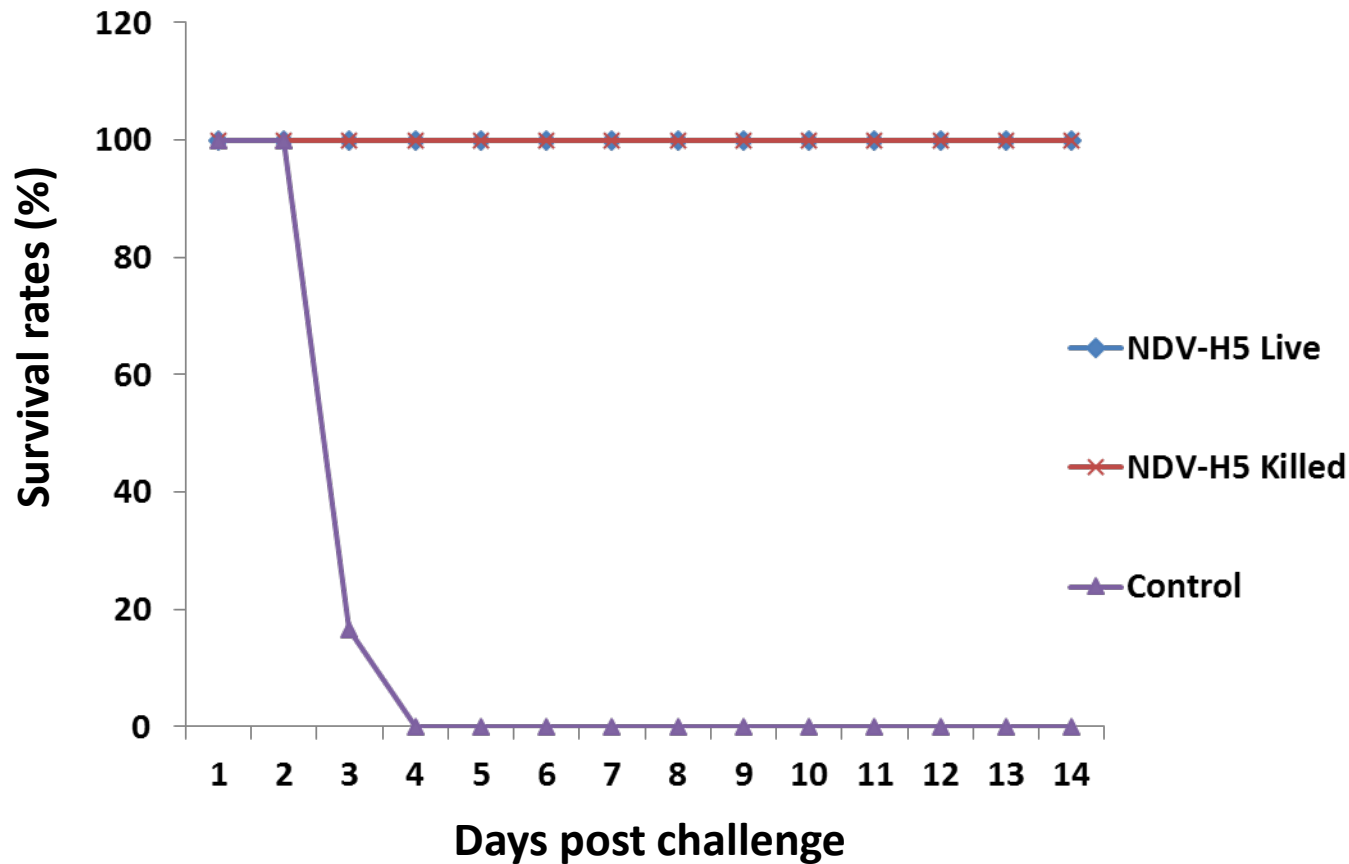
HPAIV Study in Chickens: Design



- 1) Collect blood samples at D0, D14 and D28 after vaccination;
- 2) Collect Oropharyngeal and cloacal swabs at D1, D3, D6, D9 and D14 post challenge;
- 3) Necropsy 5 or 4 chickens at D3 and D5 post challenge.



Survival rates of chickens challenged with a HPAI H5N2 virus



H5N2: A/turkey/Minnesota/9845-4/2015 (10^6 TCID₅₀)



Clinical Signs of vaccinated/mock-vaccinated chickens

A.



NDV-H5 Vaccinated chickens on 4 DPI

B.



Mock-infected chickens on 4 DPI

- No clinical signs were observed in NDV-H5 vaccinated group
- Mock-vaccinated chickens showed typical clinical signs of highly pathogenic H5N2 infection
- 100% mortality rate in control H5N2 infected chickens



Virus titers of oropharyngeal and cloacal swabs collected from chickens after challenge

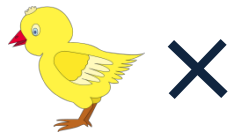
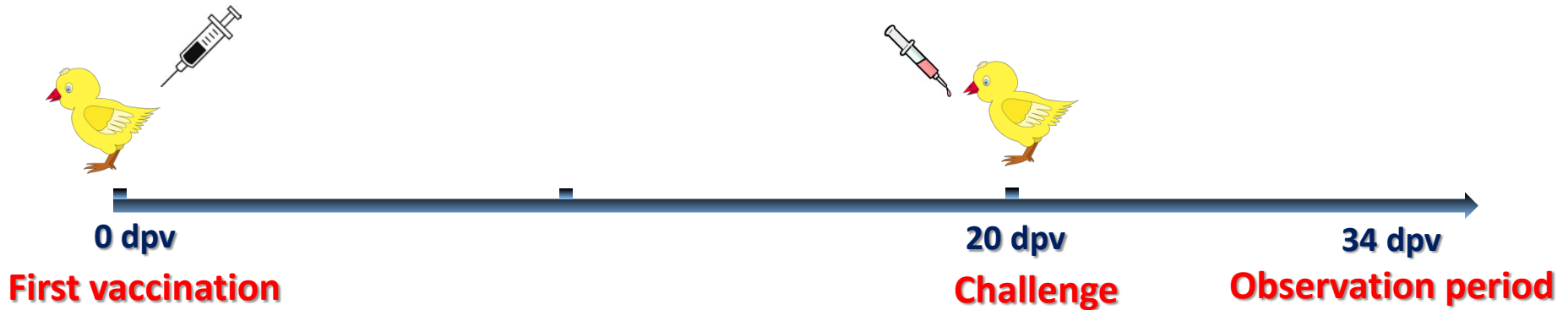
	1 dpc		3 dpc		5 dpc		7 dpc	
	Oropharyngeal	Cloacal	Oropharyngeal	Cloacal	Oropharyngeal	Cloacal	Oropharyngeal	Cloacal
NDV-H5 Live	1.70* (1/20)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
NDV-H5 Killed	2.50±0.00 (2/15)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Control	2.43±0.18 (9/15)	<1.0	3.18±0.31 (8/8)	1.70±0.00 (2/8)	N/A#	N/A	N/A	N/A

*: $\text{Log}_{10}\text{TCID}_{50}/\text{mL}$

#: all control chickens died within 5 days post challenge

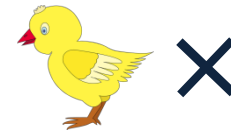


2nd Chicken Study: Evaluate Mass Application of NDV-H5 vaccine



19

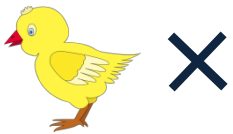
1X dose of
NDV-H5



4 and 5



H5N2: 10^6 TCID₅₀/chicken



10

Mock Control



5

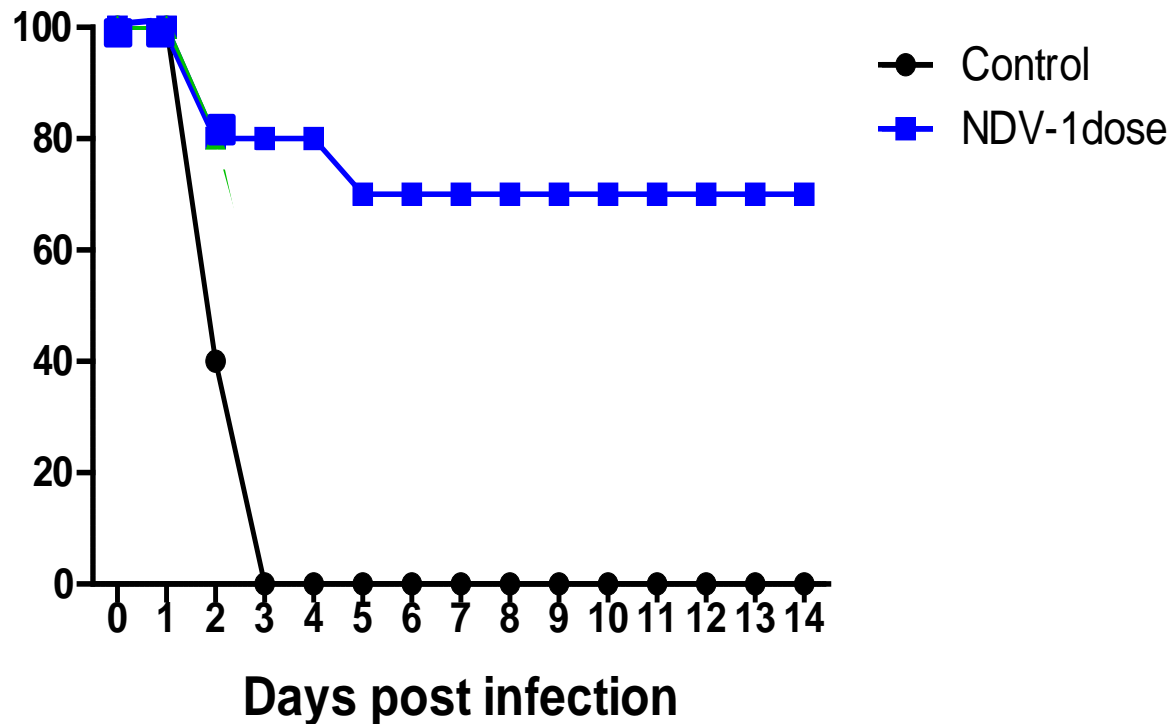
2- week old
chickens

Coarse-Spray
Vaccination

Necropsy at 2 and 4 dpc



Survival rate of coarse-spray vaccinated and H5N2-challenged chickens



- 70% of Vaccinates survived until the end of the observation period (14 DPC)
- All Controls died or were euthanized due to severe clinical signs on 3 DPC



Mass application experiment: Virus shedding

	1 dpc		3 dpc		5 dpc		7 dpc		9 dpc	
	Tracheal	Cloacal	Tracheal	Cloacal	Tracheal	Cloacal	Tracheal	Cloacal	Tracheal	Cloacal
NDV-H5 1dose	1.70* (1/19)	<1.0	2.90±0.10 (2/11)	2.80±0.50 (2/11)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Control	<1.0	2.88±0.18 (4/10)	2.50±0.33 (4/5)	2.33±0.58 (3/5)	N/A#	N/A#	N/A#	N/A#	N/A#	N/A#

*: $\text{Log}_{10} \text{TCID}_{50}/\text{mL}$

: All control chickens were dead at 3 dpi



Summary and Conclusions

- **A universal influenza vaccine for pigs seems feasible even in the presence of maternal antibodies.**
- **At this point the vaccination scheme with multiple vaccination rounds makes universal vaccines not feasible for the swine industry.**
- **Pathogenicity studies in chickens showed different virulence of the U.S. isolates of intercontinental H5Nx HPAIVs.**
- **Both NDV-H5-based live and killed vaccines completely protected chickens from clinical signs and mortality, after challenge with the HPAIV A/turkey/MN/9845-4/2015 (H5N2).**
- **Mass application of the live NDV-H5 for chickens seems feasible.**
- **NDV-H5-based live and killed vaccines are promising vaccine candidates in chickens against intercontinental H5Nx viruses suitable for mass application.**



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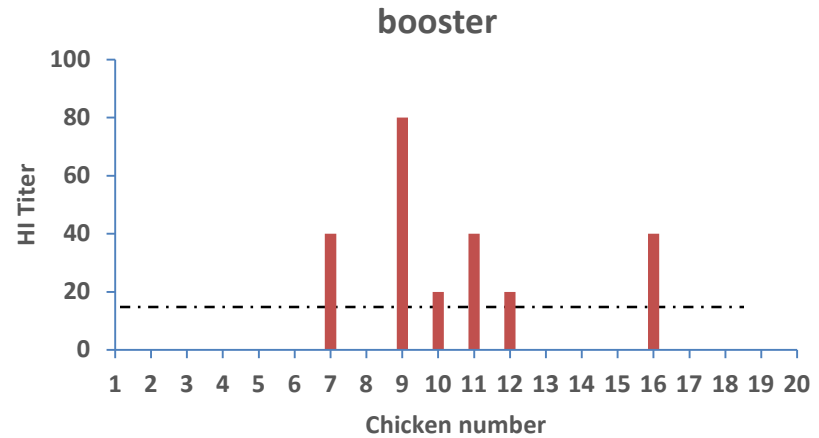
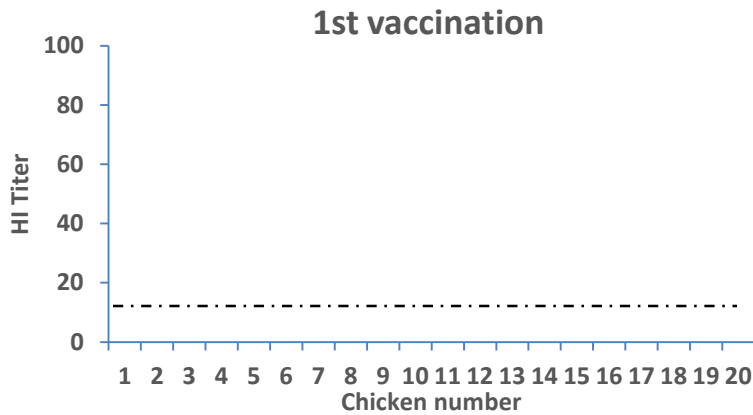
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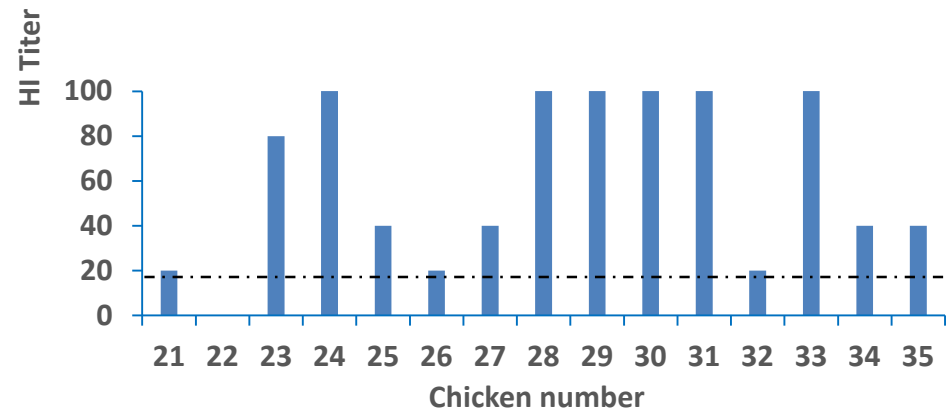
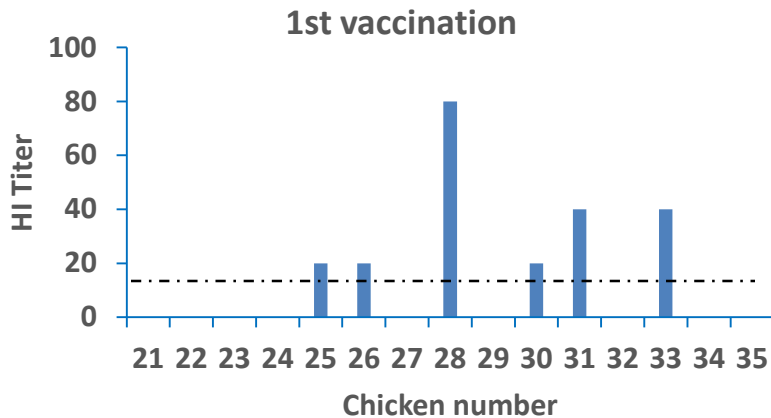


HI antibody titers of chickens vaccinated with different NDV-H5 vaccines

NDV-H5 Live



NDV-H5 killed





Virus titers of tissues collected from chickens after challenge

	Lung		Spleen		Bursa	
	3 dpc	5 dpc	3 dpc	5 dpc	3 dpc	5 dpc
NDV-H5 Live	<1.0	<1.0	<1.0	<1.0	2.50±0.00 [^] (1/5)	<1.0
NDV-H5 Killed	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Control	3.70±0.35* (7/8)	N/A [#]	2.86±0.46 (5/8)	N/A	3.67±0.36 (7/8)	N/A

*: $\text{Log}_{10} \text{TCID}_{50}/\text{mL}$

#: all control chickens died in 5 days post challenge

[^]: IHC negative: most likely cross-contamination