OFFLU avian influenza virus characterisation meeting
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Are Available Vaccines Adequate as Part of Eradication Program?

- Initial vaccine trials performed starting in January 2015 using representative H5N8 and H5N2 viruses
- Tested selected licensed vaccines or seed strains available at beginning of outbreak
  - Fowlpox vector with Turkey/Ireland/1983 insert
  - Herpesvirus of turkeys with Swan/Hungary/2006 insert
  - Licensed seed strains including Turkey/WI/1968 and TK/CA/2002 and several foreign RG vaccines
- Key determinants to measure success of vaccine
  - Protection from clinical disease
  - Reduction in viral shedding in vaccinated birds compared to controls

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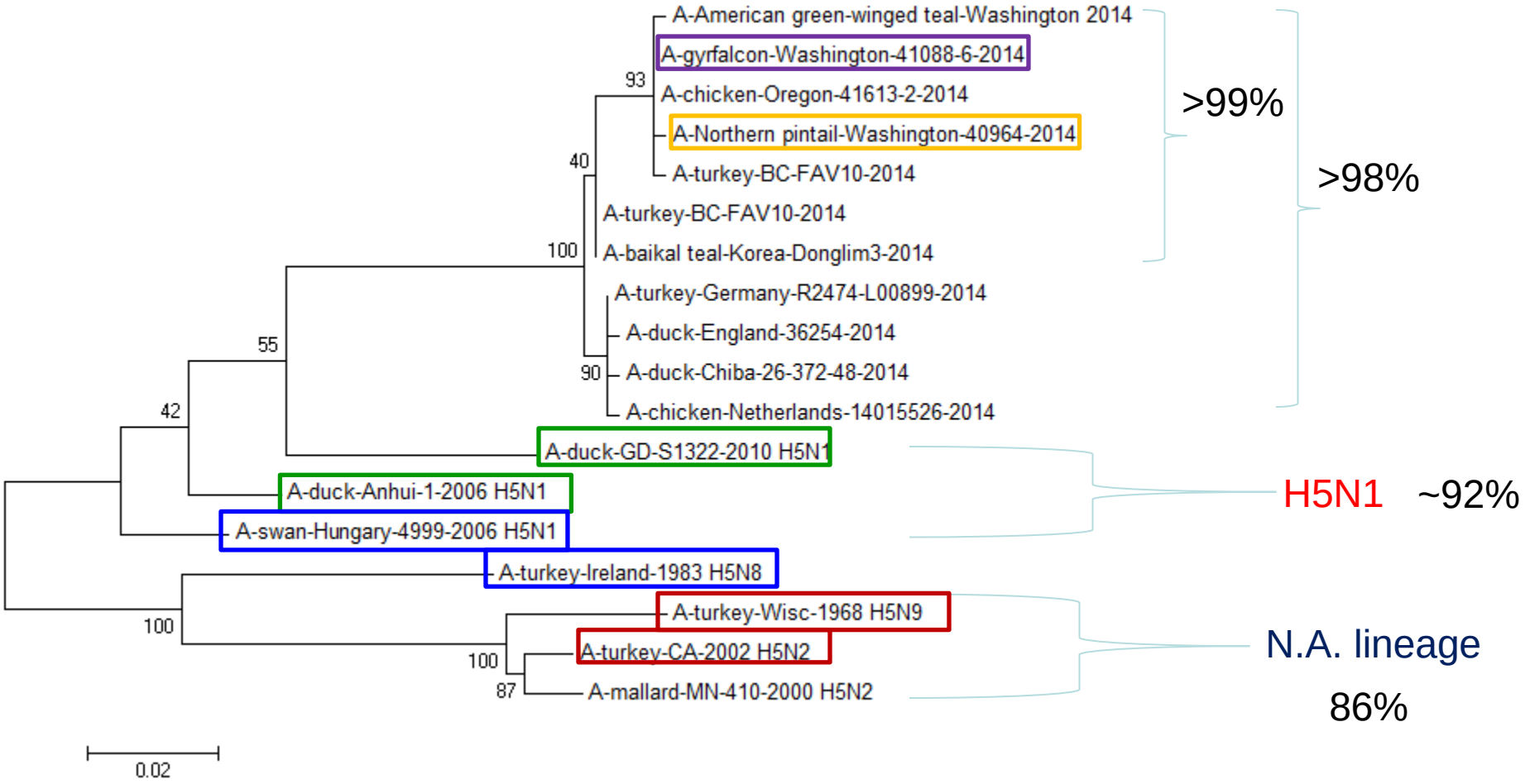
US HPAI Vaccine Studies

Notice: Vaccines studies were funded by the USDA, and USDA derives no economic benefit from the use of any of the vaccines described, and does not endorse any specific vaccine

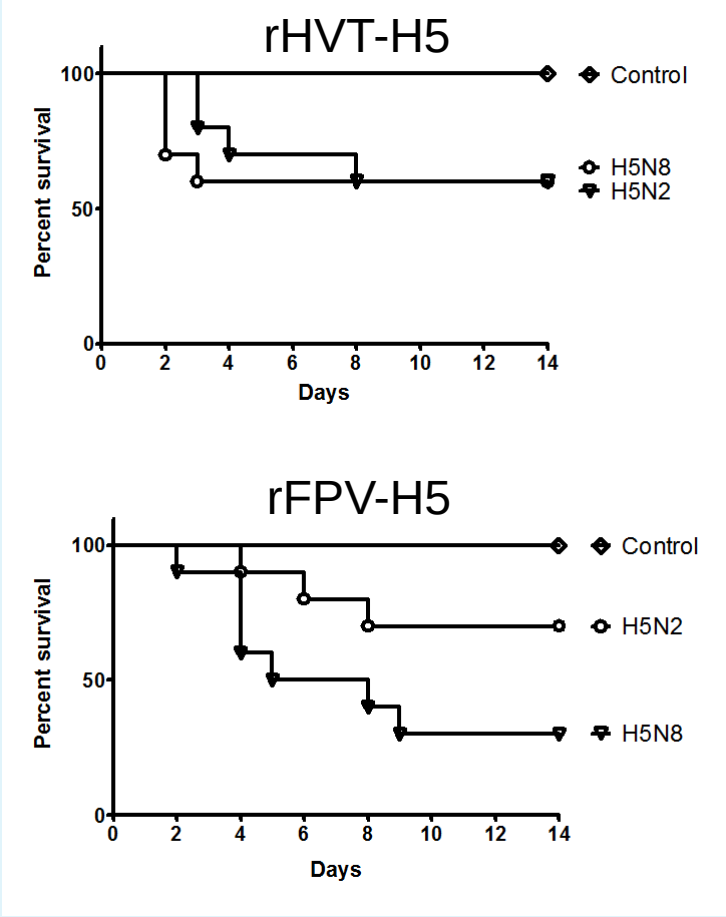
SEPRL & NVSL/NADC have completed over 25 H5 Vaccine Studies in support of outbreak

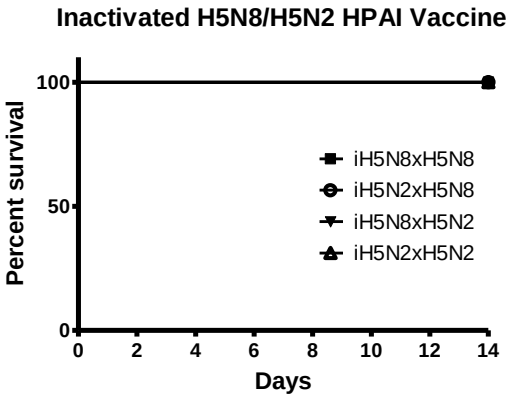
HA nucleotide sequence and phylogenetic analysis of vaccine isolates

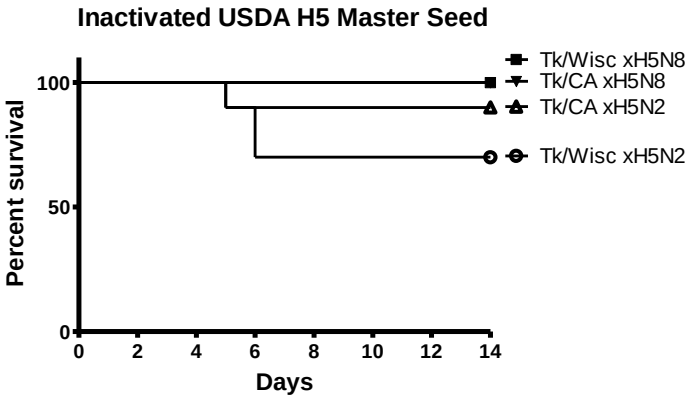


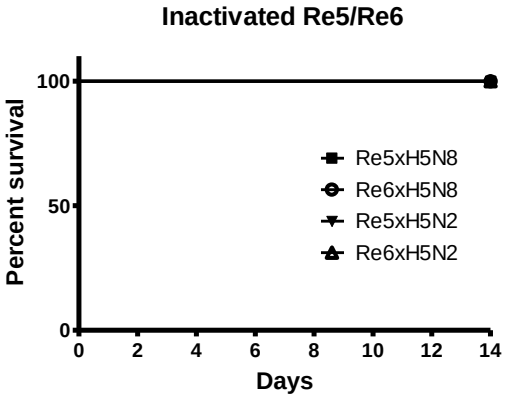
Green=RE vaccines
Blue=Commercial recombinant
Red=USDA LPAI H5 seed isolates

Mortality:


rHVT-H5
Percent survival
100
50
0
0 2 4 6 8 10 12 14
Days
Control
H5N8
H5N2
rFPV-H5
Percent survival
100
50
0
0 2 4 6 8 10 12 14
Days
Control
H5N2
H5N8


Inactivated H5N8/H5N2 HPAI Vaccine
Percent survival
100
50
0
0 2 4 6 8 10 12 14
Days
iH5N8xH5N8
iH5N2xH5N8
iH5N8xH5N2
iH5N2xH5N2


Inactivated USDA H5 Master Seed
Percent survival
100
50
0
0 2 4 6 8 10 12 14
Days
Tk/Wisc xH5N8
Tk/CA xH5N8
Tk/CA xH5N2
Tk/Wisc xH5N2


Inactivated Re5/Re6
Percent survival
100
50
0
0 2 4 6 8 10 12 14
Days
Re5xH5N8
Re6xH5N8
Re5xH5N2
Re6xH5N2

Interim Conclusions After Initial Studies

- Trials were not necessarily designed for field application
- Homologous vaccination worked the best, but you can't use HPAI as vaccine seed in U.S.!
- North American H5 seed strains provide only partial protection and not recommended alone
- Licensed viral vectors provided only partial protection when administered as single dose
- Chinese reverse genetics viruses are closer genetically to U.S. isolates and provided better protection, but unlikely to be used in U.S.
- Matching the vaccine to field virus is always recommended!
- Consider alternative vaccines

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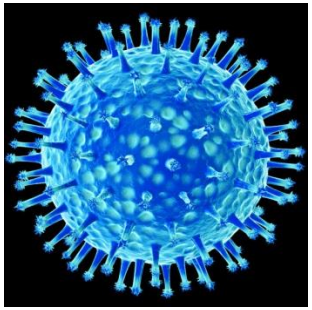
2nd Generation Vaccine Trials

- Consideration of vaccines likely to be licensed and available quickly
- Requires commitment of manufacturer to license in the absence of defined vaccine market in the U.S.
- SEPRL, with financial and technical support from APHIS, committed to evaluating the most promising vaccines because of the ongoing outbreak
- Consideration for experimental design that could be practical for field use in layer chicken, broiler chicken, or meat turkey industries
- Changed challenge strain to TK/MN/15 which was more virulent and infectious (more stringent challenge)

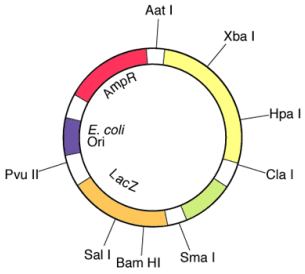
SEPRL Reverse Genetics for Vaccine Development

Highly Pathogenic Avian Influenza Virus

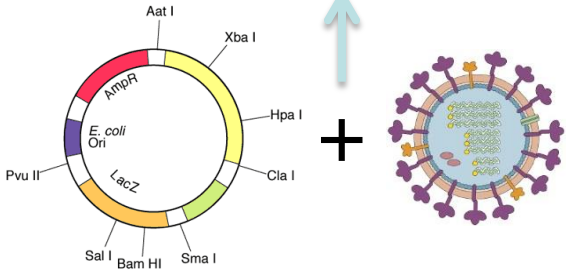


Extract RNA

RT-PCR and cloning



Modify to Low pathogenic cleavage site



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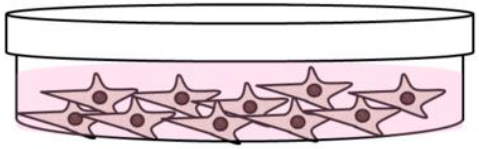
PR8 backbone



Rescue in cell culture

Grow vaccine in eggs

Inactivate virus and prepare vaccine

Vaccinate Chick!

RNA Particle Vaccine (Alphavirus)

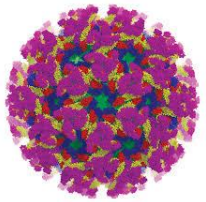
Denovo synthesize hemagglutinin gene with LP cleavage site

+

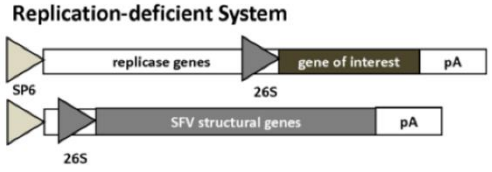
Expression plasmid

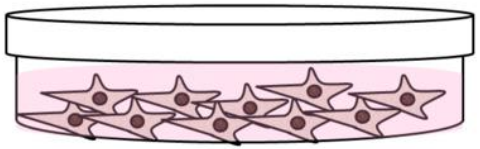
- Vaccine is replication incompetent-safety of killed vaccine
- Vector can stimulate humoral and cell mediated immune response
- Licensed platform allows rapid replacement of target gene
- Parenteral inoculation required

Vaccinate Chick!



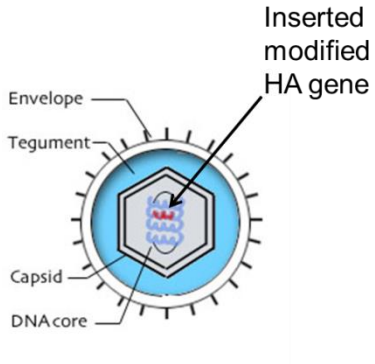
Alphavirus vector system

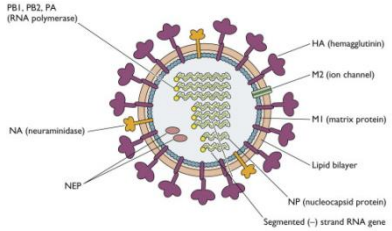


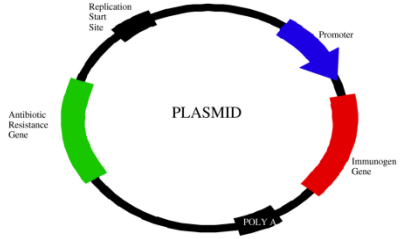


Grow in cell culture

Age of Biotechnology for Avian Influenza Vaccines







Recombinant Vectored-Live	Reverse Engineered	RNA Particle	Plasmid DNA
CEVA, Merial	Zoetis	Harris Vaccine	Benchmark Biolabs
HVT	Reverse genetics H5	Alphavirus RPH5	AIV H5 Mod DNA
FPV			

- H5-hemagglutinin genetically & antigenically match the outbreak virus
- Can rapidly change hemagglutinin gene
- Rapidly obtain conditional license for non-replicating vaccines, unclear of speed for licensing of recombinant vaccines

Results

- Homologous and the RG-Gyr Falcon killed adjuvanted vaccines provide excellent results with no clinical disease and large reduction in viral shedding after single vaccination in SPF chickens and commercial turkeys
- HVT-AI and Fowlpox vectored vaccines with partially matched hemaggutinin gene inserts had only partial clinical protection and high virus shedding
- RP studies provided good short term protection with single vaccination and strong protection with prime/boost approach
- DNA vaccine provided partial protection with 2 doses of vaccine

Vaccine-Conclusions

- Vaccine response is related both to clinical protection and viral shedding if vaccinated birds are infected
- Homologous killed vaccines provided best protection
- Other killed vaccines had good clinical protection but concerns about levels of virus shedding
- Vectored vaccines with partially matched hemagglutinin had marginal protection on their own
- Interest in being able to Differentiate vaccinated from vaccinated and then infected animals (DIVA) vaccines

DIVA

- **D**ifferentiate **I**nfected from **V**accinated **A**nimals
- **DIVA** principle primary application is to assure trading partners that livestock have not been exposed to infectious virus i.e. **differentiate vaccinated only and vaccinated and then infected poultry**
- Can also be used as surveillance tool for low virulence AIV to determine incidence of infection when vaccination is used
- Inexpensive, reliable, and high throughput differential serologic test needed to make DIVA surveillance testing viable
- For countries that do not export poultry, DIVA vaccination probably not a major priority

Summary

- Four recombinant vaccines are now licensed in the U.S.
  - HVT-AI and fowlpox-AI with heterologous insert
  - RG and RP with clade 2.3.4.4 insert
- Three of the vaccines were purchased for U.S. veterinary stockpile
- The monetary incentive of veterinary stockpile directly contributed to licensure of new vaccines

Future

- A viral vectored adenovirus serotype 9 was recently tested that also was protective
- HVT-AI vaccine has been updated with H5Nx H5 gene with improved results
- Some of the vaccines may be used in hatchery, but none of the proposed vaccines are suitable for mass administration in the field
- Serologic DIVA surveillance should be possible that may help regain export markets if vaccination is used
- Must generate data on DIVA surveillance to get internationally recognized

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, 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**
- **NVSL: Mia Torchetti**
- **NADC: Matt Sylte**