

Current Developments in AI Vaccines Including Food Safety Aspects in Vaccinated Birds



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Avian Influenza Vaccines

- **Protection:**

- **Humoral Immunity - antibodies**

- Hemagglutinin – major protection
- Neuraminidase - less protective

- **Cell-mediated immunity**

- **Innate immunity – cytokines**

- **Other Issues:**

- **Heterosubtypic immunity - anti-M2**

- **Additive immunity of multiple AI viral proteins**

- **Inactivated verses live vaccines**

Avian Influenza Vaccines

Vaccine Technologies

1. **Inactivated whole AI virus (C,E)**
2. ***In vitro* expressed HA or other proteins:**
 - **Eukaryotic tissue cultures (plant and animal), plants, yeast, bacteria and viruses (e.g. baculovirus)**
3. ***In vivo* expressed HA or other proteins:**
 - **Viruses: Fowl Poxvirus (C), adenovirus (E), VEE (E), ALV (E), vaccinia (E), ILT (E), NDV (C,E), AI-NDV chimera (E), MDV (E)**
 - **Bacterial: Salmonella (E)**
4. **Naked Nucleic acids – cDNA (E)**

Properly Used AI Vaccines

Protection – Positive Aspects

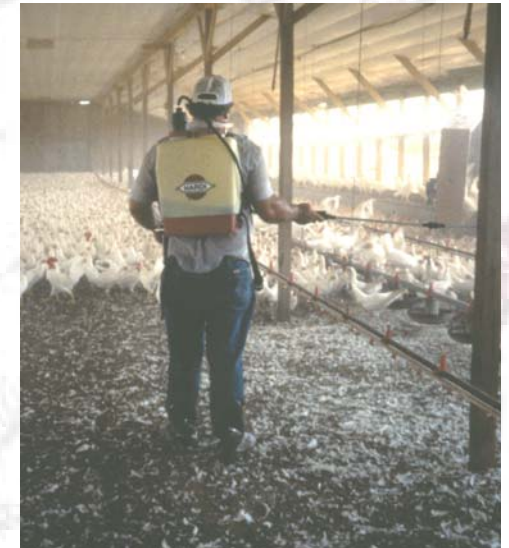
- Increase resistance to AI virus infection
- Prevent clinical signs and death
- Reduced shedding of field virus when infected
- Prevent contact transmission
- Provide long protection
- Protect against low-high exposure dose of field virus
- Protect against a changing virus, but vaccine strains have finite lifespan

Protection – Negative Aspects

- “Silent” infections
- Special virological and serological surveillance procedures and methods needed

Needs in the Next Generation of AI Vaccines

- **Mass immunization methods: water, feed, spray and *in ovo* administration**
- **Increase use of biotechnology to address genetic drift and antigen content of vaccines**
- **Vaccine combinations and protocols – e.g. AI and vector maternal antibody impact on immunization**
- **Improved adjuvants for inactivated AI vaccines in waterfowl**
- **Longer, enhanced immunity with few vaccinations**
- **Consistent quality for inactivated vaccines – including purity, safety, efficacy and potency**



Mass Immunization

Recombinant Adenovirus-AIV H5: SQ and IN

Chickens vaccinated SQ or IN at 3 wks with $10^{10.6}$ d-Adenovirus-AIV-H5 (VN/1203/04) recombinant* and IN challenged at 6 wks with 10^6 EID₅₀ of HPAIV A/Vietnam/1203/2004 [H5N1]

Vaccine Group	Morbidity	Mortality (Mean Death Time in days)	Virus Isolation, 2 days Post-challenge (Log10 EID50 titer/ml)	
			Oral swab	Cloacal swab
SQ: d-Adenovirus Vector	10/10	10/10 (1.8)	10/10 (6.96)	10/10 (6.26)
SQ: d-Adenovirus-AIV-H5	0/10	0/10	9/10 (3.84)	0/10 (^a 0.9)
IN: d-Adenovirus Vector	10/10	10/10 (1.8)	NA	NA
IN: d-Adenovirus-AIV-H5	5/10	5/10 (6.0)	NA	NA

- **Some virus vectors (such as FP and Adenovirus) work parenterally and not topically (IN, IT, eyedrop)**

Mass Immunization

Recombinant Adenovirus-AIV H5: *In Ovo*

Chickens vaccinated in ovo at E18d with $10^{8.2}$ Adenovirus-AIV-H5 (A/turkey/WI/68) recombinant*, revaccinated IN at 15d and IN challenged at 34d with 10^5 EID₅₀ of HPAIV A/chicken/Queretaro/14588-19/95 [H5N2])

Vaccine Group	Mortality	HI Serology D29 (GMT)
Unvaccinated	11/11	-
d-AdTW68.H5 - E18d	0/7	7
AdTW68.H5 - E18, 15d	0/12	6.1

Mass Immunization

Inactivated AI Vaccine: *In Ovo*

Chickens vaccinated in ovo at E18d with inactivated Tk/WI H5N2 (oil emulsion) and IN challenged at 34d with 10^7 EID₅₀ of HPAIV A/chicken/Pennsylvania/1370/83 [H5N2]

Vaccine Group	Mortality	HI Serology D24 (GMT)
Unvaccinated	5/5	0/5 (<10)
Tk/WI	0/8	12/14 (1194)

Stone et al., Av. Dis. 41:856-863, 1997

- **Future: Impact mixing inactivated vaccines with live MDV**
- **MDV vectored for *in ovo* use**

Mass Immunization

Recombinant NDV-AI-H7 Vaccine: Eyedrop

Chickens vaccinated at 2 and 4 weeks-of-age with rNDV-AI-H7 or B1 parent virus vaccines (eyedrop), and IN challenge at 6 weeks-of-age with 10^5 EID₅₀'s of vvNDV (Fontana/73) or A/Steele/59 (H7N7)

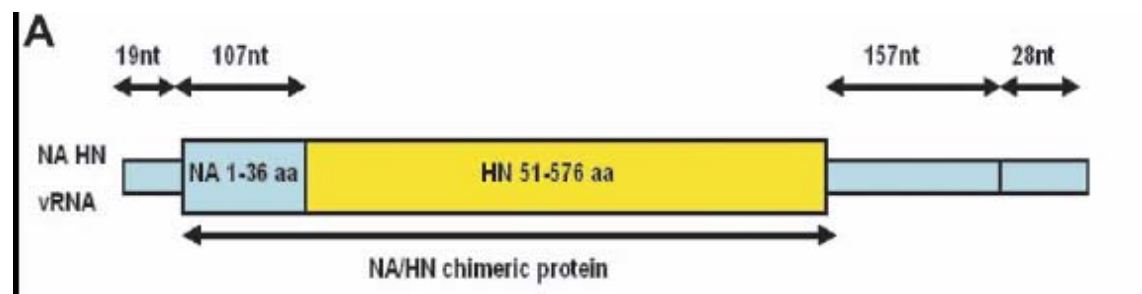
Vaccine Group (10 birds/group)	Challenge	Morbidity (≥ 3)	Mortality (MDT)	Survivors (%)
B1H7, 1x	Fontana	0/10	0/10	100
B1H7, 1x	Steele	1/10	1/10 (4.-0)	90
B1H7, 2x	Fontana	0/10	0/10	100
B1H7, 2x	Steele	1/10	1/10 (7.0)	90
SEP B1, 2x	Fontana	0/10	0/10	100
SEP B1, 2x	Steele	10/10	10/10 (3.3)	0
Sham, 2x	Fontana	10/10	10/10 (4.0)	0
Sham, 2x	Steele	7/10	7/10 (2.3)	30

Mass Immunization

- **NDV-AI-H5:**
 - **BHG/QH/05 – 100% chickens protected from homologous challenge and GD/96 (Ge et al. JVI 81:150-8, 2007)**
 - **N.Amer H5 – 90% chickens protected from Queretaro/95 (H5N2) – (Poster this conference)**
 - **ck/Italy/98 – 100% chicken protection from homologous virus (H5N2) (Veits et al. PNAS 103:8197-8202, 2006)**
- **ILT-AIV-H5:**
 - **ck/Italy/98 – 100% protection in chickens from homologous and Ck/Scotland/59 (Vaccine Luschow et al., 19:4249-4259, 2001)**

Mass Immunization

- **Salmonella vector** – avirulent deleted mutants, licensed for use as live vaccine in USA; potential as a vector for AI genes (Poster this meeting)
- **AI vectored vaccine** – reduced virulence (point mutations or partial deletions in some genes) - restrict replication in host: ex. cold-adapted vaccines
- **AI-NDV Chimera** – AI virus with replacement of part of AIV NA with part of NDV HN (Park, M.S., et. al, Proceedings of the National Academy of Science 103(21):8203-8208, 2006)



Biotechnology to Solve Genetic Drift: H5

- Fowl pox with H5 AIV gene insert
- Different challenge viruses (87.3-100% aa sequence similarity)



* challenge viruses

(Updated from Swayne et al., Vaccine 18:1088-1095. 2000)

Biotechnology to Solve Genetic Drift: H5

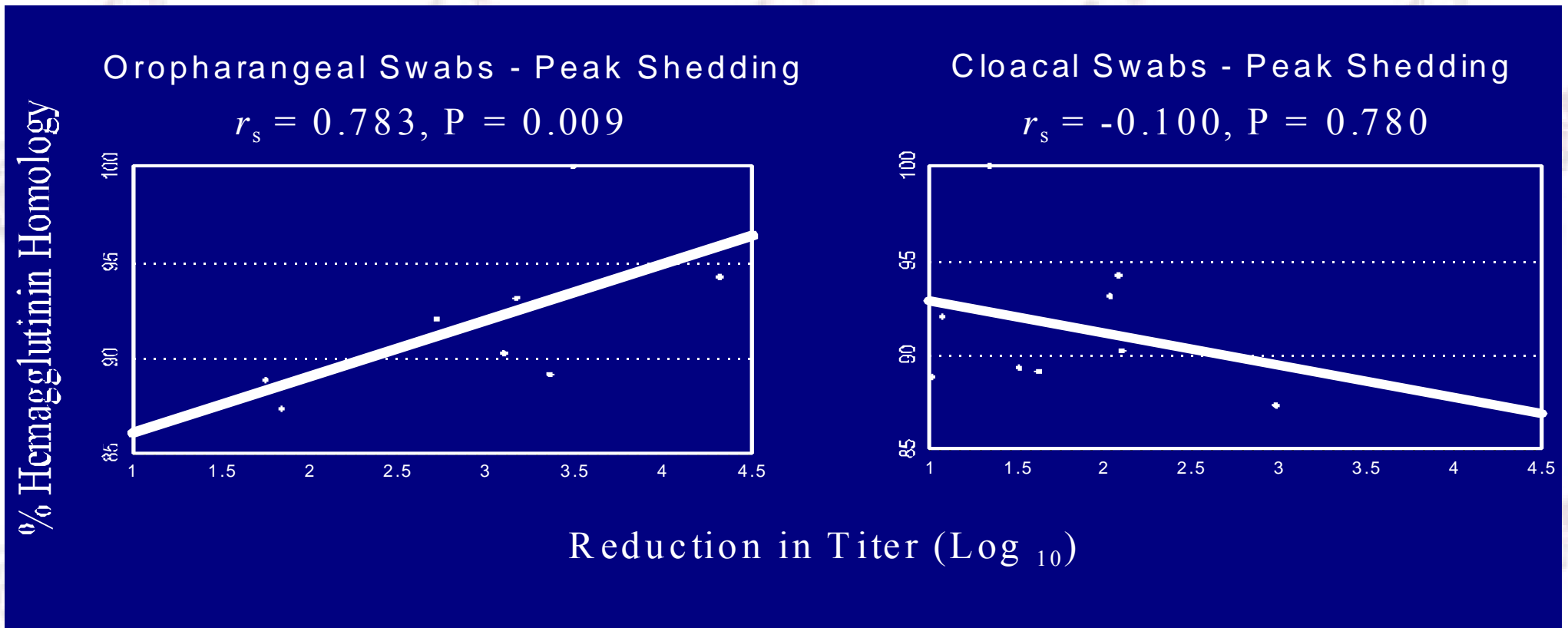
Chickens vaccinated SQ 1d with rFP-H5-Ire/83 and IN challenged at 3 wks with 10^{5-6} EID₅₀ of HPAIV

Challenge Virus	Subtype	HA Similarity to Tk/Ire/83	Mortality (MDT)
Ck/Scotland/59	H5N1	92	0/10
Tern/S. Afr/61	H5N3	93.1	0/10
Tk/Ontario/66	H5N9	89.1	0/10
Ck/PA/83	H5N2	87.3	0/10
Tk/Ireland/83	H5N8	100	0/10
Tk/England/91	H5N1	94.2	0/10
Ck/Queretaro/95	H5N2	89.3	0/10
HK/156/97	H5N1	90.2	0/10
Ck/S. Korea/03	H5N1	89.9	0/10
ck/Vietnam/04	H5N1	88.4 est	0/22
WS/Mongolia	H5N1	89.7	1/8
Control	-	-	80-100%

- **Excellent protection from mortality using diverse H5 AIV**

Biotechnology to Solve Genetic Drift: H5

- Variable reduction in shedding of challenge virus



(Swayne et al., Vaccine 18:1088-1095. 2000)

Biotechnology to Solve Genetic Drift: H5

- **H5 AI Vaccine in Mexico since 1995**

Challenge virus	DPI ^a	Oropharyngeal Viral Titers	
		Vaccinated	Control
Vaccine strain	3DPI	1.66 (5/10)	4.5 (5/5)
Jalisco Lineage	5DPI	0.98 (4/10)	3.1 (5/5)
CK/AG/124-3705/98	3DPI	4.44 (10/10)	4.2 (5/5)
Lineage A	5DPI	2.14 (8/10)	2.4 (5/5)
CK/Guatemala/194573/02	3DPI	4.86 (10/10)	4.9 (5/5)
Lineage B	5DPI	3.62 (10/10)	3.4 (5/5)

- **1998 and 2002 Central American H5N2 LPAI viruses have drifted away from vaccine viruses**

Biotechnology to Solve Genetic Drift

H7 AIV

Chickens vaccinated SQ at 1d with rFP-H7-Vic/85 and IN challenged at 3 wks with $10^{7.1}$ EID₅₀ of HPAIV A/chicken/Pakistan/1369-CR2/95 [H7N3]) or A/turkey/Italy/4580/99 (H7N1)

Vaccine/ Challenge Virus	Morbidity (2-4+)	Mortality (MDT)	HI Serology					
			Pre-Challenge (GMT)			Post Challenge (GMT)		
			Vic/85	Italy/99	Pak/95	Vic/85	Italy/91	Pak/95
rFP-H7/ Italy	9/10	9/10 (2.8)	6/10 (8)	0/10	0/10	1/1 (320)	1/1 (320)	NA
rFP-H7/ Pakistan	1/10	0/10	9/10 (12)	0/10	0/10	10/10 (57)	NA	10/10 (46)
FP/ Italy	10/10	10/10 (2.8)	0/10	0/10	0/10	NA	NA	NA
FP/ Pakistan	10/10	10/10 (4.4)	0/10	0/10	0/10	NA	NA	NA

Biotechnology to Solve Genetic Drift

H7 AIV

Chickens vaccinated SQ 1d with recombinant fowlpox vaccines and IN challenged at 3 wks with 10^6 EID₅₀ of HPAIV A/turkey/Italy/4580/1999 [H7N1])

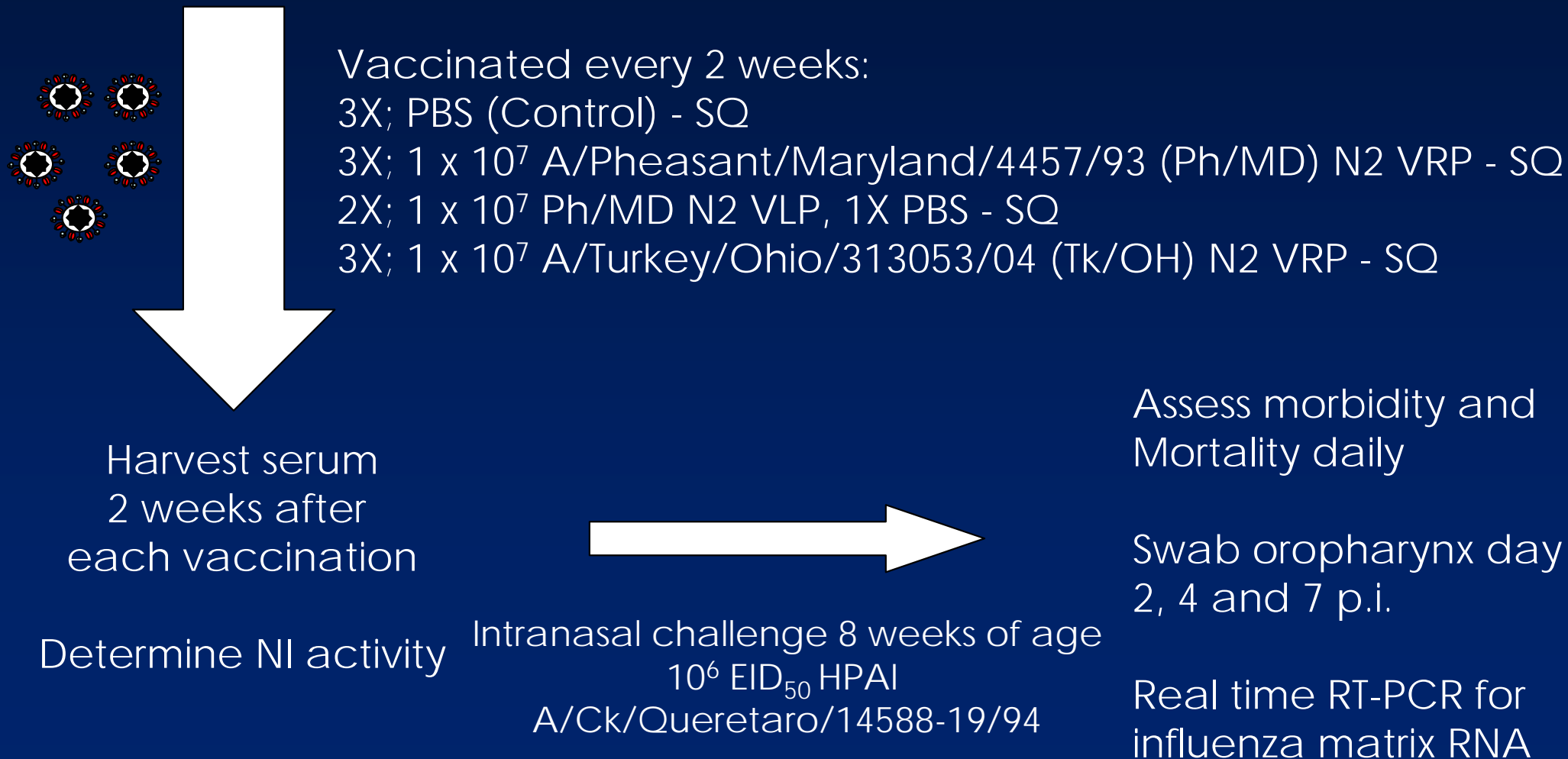
Vaccine Group	Morbidity	Mortality (MDT)	HI Serology (GMT)		Virus Isolation(Log ₁₀ EID ₅₀ titer/ml)	
			Pre	Post	oral	cloacal
rFP-H7-Vic/85	8/10	8/10 (4.0)	1/10 (5)	2/2 (1024)	ND	ND
rFP-H7-It/00	1/10	1/10 (5.0)	8/10 (10)	9/9 (776)	6/10 ^a (2.1 ^A)	2/10 ^a (1.3 ^A)
rFP-F7-VA/02	8/10	8/10 (3.3)	4/10 (7)	2/2 (2048)	ND	ND
rFP-N1-It/00	8/10	7/10 (5.0)	-	3/3 (128)	ND	ND
rFP	10/10	10/10 (2.3)	0/10	NA	10/10 ^a (6.7 ^B)	9/10 ^b (3.5 ^B)

- Continental lineages of H7 AIV with variation in cross protection with H7 HA subtypes
- NA gave poor protection when used alone in this study

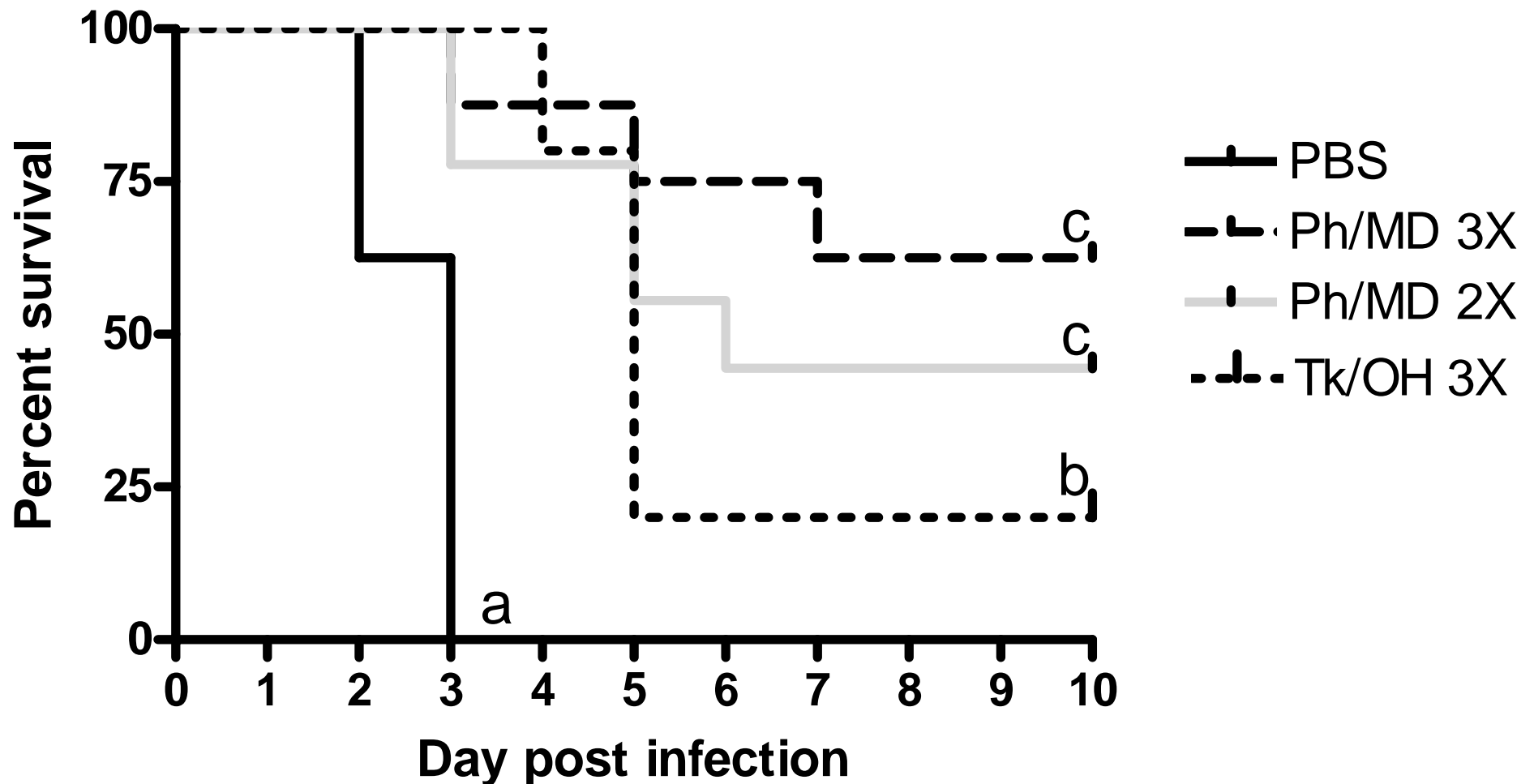
Biotechnology to Solve Genetic Drift

Neuraminidase as Immunogen

2 weeks old SPF White Leghorn chickens



Multiple vaccinations with VRP expressing Ph/MD N2 significantly increase survival rates from challenge with A/Ck/Queretaro/95 (H5N2) HPAIV



Sylte et al, Vaccine, in press, 2007

Different letters indicate significant difference in survival rate ($P < 0.05$) as compared to PBS control; Logrank test

Application

- 1. Cassette concept for licensing and use**
 - **Reverse genetics produced inactivated AIV strain: change HA in vaccine virus backbone**
 - **Replaceable cassette for virus and bacterial vectors as live or killed vaccine – FP, etc.**
- 2. Better understanding and protocols for using vectored vaccines**
 - **Need to know the impact of maternal antibodies or active immunity against the vector or AIV on replication of recombinant; ex. NDV**
 - **Species susceptibility to vector**

H5N1 HPAI

- **H5N1 HPAI has not been a Food Safety issue**
- **Exposure Risks for Human Infection**
 - **Exposure 1 week before illness to live poultry, especially direct handling of sick or dead poultry**
 - **Young age**
 - **Limited human-to-human transmission**
 - **Types of Poultry: Village or Live Poultry Market**
- **Possible exposure mechanisms: aerosols, direct hand to mouth transplant, consumption of raw products?**
- **HPAI is a trade issue – impact imports**
 - **Mounts et al., J. Inf. Dis, 180:505-508, 1999**
 - **Bridges et al., J. Inf. Dis. 185:1005-1010, 2002**
 - **Tran et al., NEJM 350 [12]:1179-88, 2004**
 - **Chotpitayasunondh et al., EID 11(2):201-9, 2005**

Can AI virus be present in poultry meat?

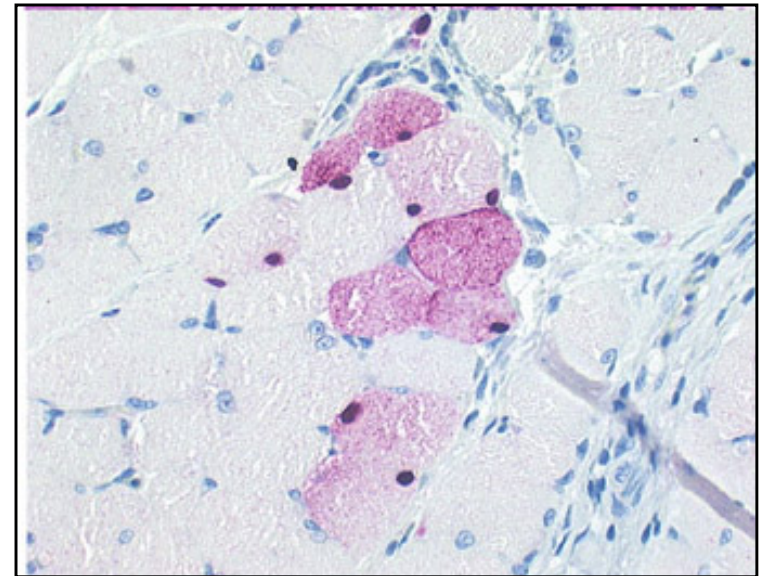
Exp.: LP & HPAIV in Chickens

Virus	Resp.	GI	Blood	Bone	Meat	Oral Trans.
H7N2/99 LPAI	++ 1-5d	+ 5d	-	-	-	-
H7N2/02 LPAI	+++ 1-7d	++ 2-7d	-	-	-	-
H5N2 HPAI*	++++ 1-5d	++++ 1-5d	+++ 1-5d	+++ 1-5d	+++ 1-5d	-
H5N1 HPAI**	ND	ND	ND	ND	++++ 1-2d	+ 90%

- **Conclusion: HPAIV can be in chicken meat?**

Demonstration of H5N1 in Meat

- **Experimentally: bird isolates (2003-5 S. Korean, Thailand. Mongolia and Indonesian), human isolates (2004-5 Indonesia, Vietnam & Thailand)**
 - **Chickens**
 - **Ducks**
 - **Japanese Quail**
 - **Geese**
- **Natural infections:**
 - **Raw frozen duck meat, 2001 and 2003**



Tumpey et al., J. Virol. 76(12):6344-55, 2002
Mase et al. Virol 339:101-9, 2005

Can vaccination prevent HPAI virus in meat?

Challenge with A/chicken/Korea/ES/03 (H5N1) 3 wks after vaccination

Group	Virus isolation from meat (Log ₁₀ EID ₅₀ /gm)		Virus Dose/Bird (Log ₁₀ EID ₅₀)
	Breast	Thigh	
Fowlpox-AIV-H5 vaccine	- ^A	-	ND ^B
Inactivated vaccine	-	-	ND
Sham	7.3	ND	7.8

^A- = negative on virus isolation, ND = not done

10 SPF WL fed the meat – 9 of 10 died

Swayne & Beck, Avian Dis. 49(1):81-85, 2005

- Inactivated AI vaccine in domestic ducks prevented dk/VN/05 (H5N1) HPAIV in meat, blood and viscera**

(Beato et al. Vaccine, epub Feb 8, 2007)

Conclusions

- 1. Vaccine have been a valuable tool in the control of AI, primarily inactivated AIV and recombinant fowlpox-AIV-H5**
- 2. Mass immunizing vectors will provide improved application method for control of AI**
 - a) Increase ease of application and reduce cost**
 - b) Need to develop vaccination protocols to optimize usage – overcome maternal or existing active immunity, improved application methods, in ovo usage by industrial sector, etc.**

Conclusions

- 3. Genetic drift will continue and will challenge us to use biotechnology to produce effective vaccines faster**
- 4. Immunization can prevent or reduce replication of HPAI virus in meat, eggs and viscera of poultry giving an extra layer of safety in control**

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