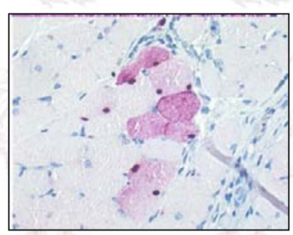
Potential Role of Exposure to Poultry Products and By-products for Human H5N1 infections

David E. Swayne, A. Lipatov, Y.K. Kwon, M. Jackwood & J. Beck
USDA/Agricultural Research Service
Southeast Poultry Research Laboratory
Athens, Georgia

Introduction

- HPAIV – systemic infection with virus in meat, bone, organs and inside eggs
  - Natural infections - imported raw duck meat from China into S. Korea (2001) and Japan (2003)
  - Experimental studies: chickens, ducks, Japanese quail, geese
  - Viruses: Eurasian-African H5N1 viruses, N. Amer. H5N2, Eurasian H7N7
- Virus demonstrated by:
  - Virus isolation
  - RRT-PCR
  - IHC



Virus Titers in H5N1 HPAIV Infected Meat and Eggs

- **Meat: H5N1 HPAIV**

Tumpey et al., J Virol 76:6344-6355, 2002
Swayne & Beck, Av Dis 49:81-85, 2005
Swayne, Int J Food Micro 108:268-271, 2006
Thomas & Swayne, J Food Prot 70:674-680, 2007
Das et al., Av Dis 52:40-48, 2008

Species	Clinical Features	Titer (EID50/g)
Chicken	Dead	5.6-8.0
	Sick	4.0-7.4
	Non-sick	1.9-5.7
Duck	Sick	4-6
	Non-sick	2.8

- **Eggs: 1983 H5N2 HPAIV from USA**

	No.+/total (log10 EID50/ml)			
	1d	**2d**	**3d**	**4d**
Egg Shell	0/15	10/16(3.6)	6/6(3.4)	-
Albumen	0/15	11/16(3.2)	6/6(3.9)	-
Yolk	0/15	10/16(1.8)	6/6(3.5)	-

Swayne & Suarez, Develop. Biol. 130:121-131, 2008

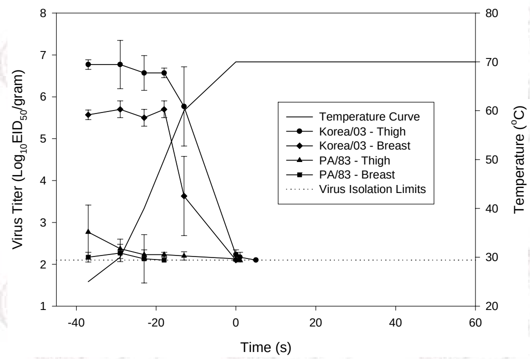
Pasteurization to Inactivate HPAIV at Levels Present in Eggs from Infected Hens

- **Pasteurization at industry standards for temperature and times for salmonella inactivation were effective at inactivation of HPAIV in liquid products**
  - **Questions as to dried egg white – moisture content was not controlled in original test**
- **Recent work with 6.5-8% moisture in dried product indicated pasteurization was effective at industry standards – ex. 2.6 days at 54.4°C**

Swayne & Beck, Av. Pathol. 33(5):512-518, 2004
Swayne & Thomas, Avian Influenza, Wylie-Blackwell, pp. 493-506, 2008

Methodology to Demonstrate Kill of HPAIV in Meat

- **Cooking 70ºC, last detection < 5s treatment, calculated endpoint 5.5s**



Swayne, Int. J. Food Microbiol, 268-271, 2006
Thomas & Swayne, J. Food Protect. 70:674-680, 2007
Thomas & Swayne, J. Food Protect, 71:1214-1222, 2008

Vaccination to Prevent HPAIV in Meat

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

Group	Virus isolation from meat (Log_{10} EID_{50}/gm) Breast	Thigh	Virus Dose/Bird (Log_{10} EID_{50})
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)

Vaccination to Prevent HPAIV in Eggs

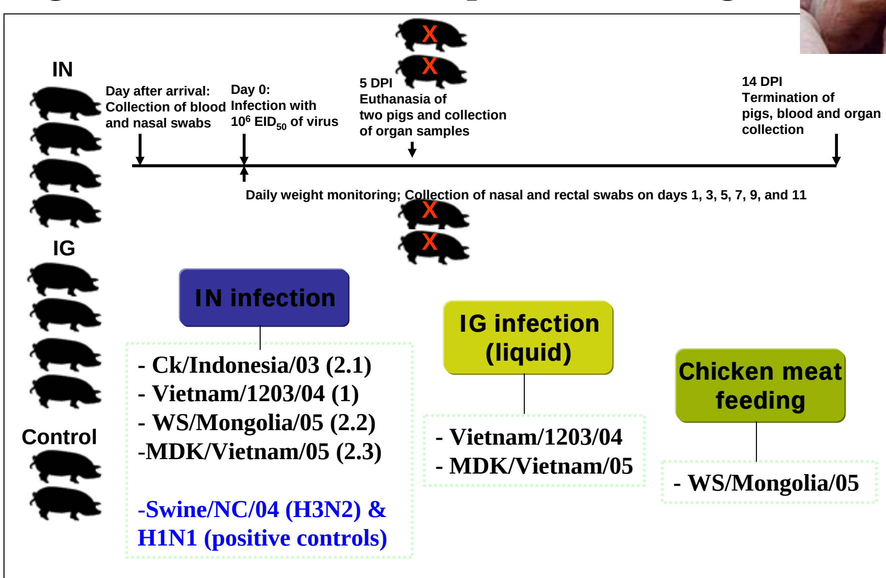
- **Single killed H5 AI or sham vaccine**
- **Challenge with A/Pennsylvania/1370/83 (H5N2) HPAIV**

SHAM	**No.+/total (log10/ml)**			
	1d	**2d**	**3d**	**4d**
Egg Shell	0/15	10/16(3.6)	6/6(3.4)	-
Albumen	0/15	11/16(3.2)	6/6(3.9)	-
Yolk	0/15	10/16(1.8)	6/6(3.5)	-

AI Vax	**No.+/Total (log10/ml)**			
	1d	**2d**	**3d**	**4-14d**
Egg Shell	0/13	3/14(1.23)	3/20(1.32)	0/72
Albumin	0/13	0/14	0/20	0/72
Yolk	0/13	0/14	0/20	0/72

Swayne & Suarez, Develop. Biol. 130:121-131, 2008

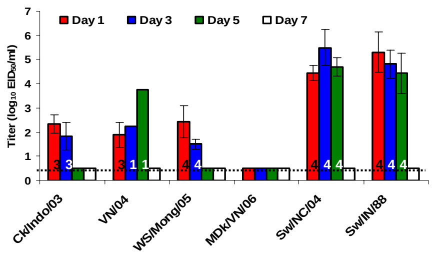
Pig Transmission Model: Experimental Design



3-weeks-old a Land Race x Large White cross male castrated piglets were used in the experiments.

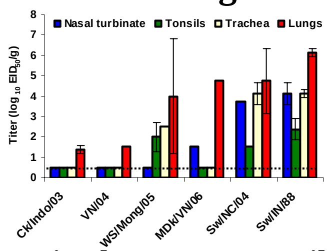
(Lipatov et al., PLOS Pathogens 7:e1000102, 2008)

H5N1 HPAIV & H3N2/H1N1 SIV: IN Inoculation



- Mild weight loss: WS/Mong/05, Ck/Indo/03 H5N1 and H1N1 SIV
- WS/Mong/05, Ck/Indo/03 and VN/04 in nasal swabs, mainly days 1 & 3
- Titers of H5N1 viruses lower than SIV

Virus shedding and organ titers on 5 DPI



- 4 H5N1 replicated respiratory tract
- Lung titers of 2 H5N1 viruses were lower than SIV, 2 HPAIV similar SIV
- WS/Mong/05 virus was isolated from trachea and tonsils, and MDk/VN/05 virus was isolated from nasal turbinate
- Histology: H5N1 - mild to moderate bronchiolitis and multifocal alveolitis. SIV, severe tracheobronchitis and bronchointerstitial pneumonia

(Lipatov et al., PLOS Pathogens 7:e1000102, 2008)

H5N1 HPAIV: IG inoculation

- Liquid media (10^6 EID_{50} WS/Mong./05 & VN/1203/04), 2 viruses – no evidence of infection
- Feeding WS/05 infected meat (100g, 10^{10} EID_{50})

Virus titers in nasal swabs on day 3 (log_{10} EID_{50}/ml) [a]	Organ titers on day 5 (log_{10} EID_{50}/ml) [b]		Serum antibody titer (VN test) to WS/Mong/05 virus	
	Nasal turbinate	Tonsils	Pre-exposure	Post-exposure
2.63±0.49	2.63±0.18	2.88±0.18	<20	80

- No lesions
- No weight loss
- No changes in food consumption
- No behavioral abnormalities
- No virus rectal swabs or GI tissues

- Transmission by consumption of infected meat
- Respiratory infection, no evidence of digestive infection

(Lipatov et al., PLOS Pathogens 7:e1000102, 2008)

Ferret model: Experimental Model

2 ferrets euthanatized 5 DPI
Organs for histological and virological examination

Daily monitoring of temperature; Measuring of body weight and collection of nasal washes and rectal swabs on days 1, 3, 5, 7, 9 and 11

Terminated 15 DPI
Serum collected

12-20-weeks-old female ferrets, groups of 4 were inoculated
IN (10^6 EID_{50}/0.5 ml) or IG (10^6 EID_{50}/2 ml)

IN infection	IG infection (liquid)	Chicken meat feeding	IG infection (grind)
Ck/Indonesia/03 VN/1203/04 WS/Mongolia/05 MDk/Vietnam/05	Ck/Indonesia/03 VN/1203/04 WS/Mongolia/05 MDk/Vietnam/05	VN/1203/04 WS/Mongolia/05 MDk/Vietnam/05	VN/1203/04

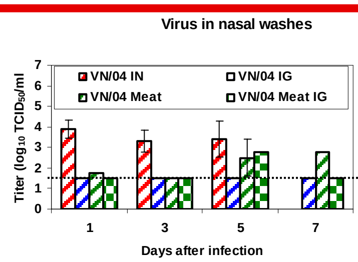
(Lipatov et al, JID in press)

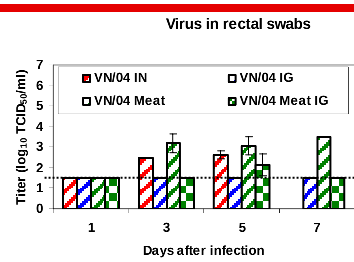
Ferret Model: Results

- IG virus in liquid: no infection
- IN virus in liquid:
  - Ck/Indonesia/03 & MDk/Vietnam/05 - mild or asymptomatic respiratory infection
  - WS/Mongolia/05 – Severe respiratory inf. (nasal washes, turbinates & lung) & inf. of olfactory bulb
  - Vietnam/04 – systemic disease including respiratory and digestive systems (including liver & pancreas)
- Meat consumption:
  - MDk/Vietnam/05 – mild respiratory infection
  - WS/Mongolia/05 – infection & mild respiratory disease: 2 dpe – virus in tonsil and nasal turbinates, 5 dpe – virus negative

(Lipatov et al, JID in press)

VN/04 Virus Exposure Route on Infection





- 5 DPI – virus titer
  - \- = no detection
  - \+ = 1.5-2.5
  - ++ = >2.5-3.5
  - +++ = >3.5

System	IN	IG	Meat	Meat-IG
Tonsil	+	-	+	-
Respiratory				
Nasal Turb	+++	-	+++	++
Lung	+++	-	+	-
Digestive				
SI	-	-	+	+
LI	+	-	+++	++
Liver	+	-	+++	+++
Systemic				
Spleen	+	-	+	+
Brain	++	-	+	+
Olf bulb	+	-	-	-

(Lipatov et al. JID in press)

Summary

- HPAIV is contained in infected poultry meat and eggs
- Cooking and pasteurization of infected meat inactivated HPAIV while freezing (-20C) is unlikely to produce predictable inactivation
- In the pig model, consumption of WS/05 H5N1 infected meat caused respiratory infection via tonsil and pharynx exposure
- In ferret model, consumption of MDk/05 and WS/05 caused non-fatal respiratory infection while VN/04 caused lethal infection with initiation of infection in both respiratory and digestive tracts

Summary

- Based on animal models, the potential for human infection would require consumption of raw HPAIV infected meat at higher doses of virus than through respiratory exposure, and intestinal component maybe virus strain dependent
- Mitigation steps: 1) cooking or pasteurization, 2) vaccination of poultry to prevent viremia and organ/muscle infection

Thank You For You Attention!

22

Trade and Food Safety Aspects for Avian Influenza Viruses

David E. Swayne and Colleen Thomas