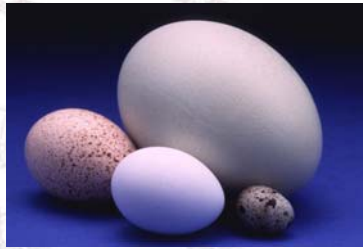


Potential Role of Exposure to Poultry Products and By-products for Human H5N1 infections



Southeast
Poultry
Research
Laboratory

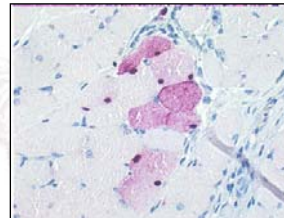


David E. Swayne, A. Lipatov, Y.K. Kwon, M. Jackwood & J. Beck

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
Duck	Non-sick	1.9-5.7
	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)	-

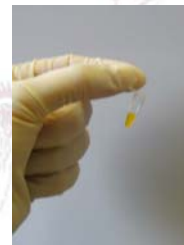
Swayne & Suarez, Develop. Biol. 130:121-131, 2008

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

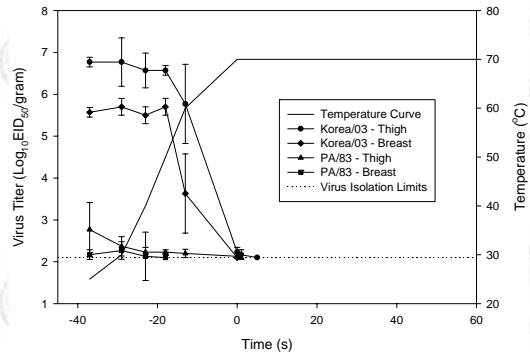
• Recent work with 6.5-8% moisture in dried product indicated pasteurization was effective at industry standards – ex. 2.6 days at 54.4°C



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

Methodology to Demonstrate Kill of HPAIV in Meat

- Cooking 70°C, 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 ($\text{Log}_{10} \text{EID}_{50}/\text{gm}$)		Virus Dose/Bird ($\text{Log}_{10} \text{EID}_{50}$)
	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)

Vaccination to Prevent HPAIV in Eggs

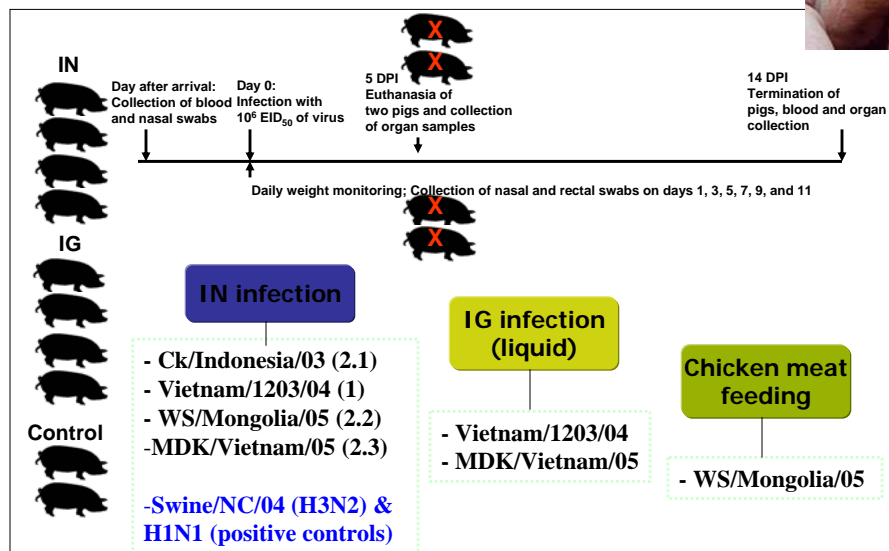
- Single killed H5 AI or sham vaccine
- Challenge with A/Pennsylvania/1370/83 (H5N2) HPAIV

SHAM	No.+/total (log ₁₀ /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 (log ₁₀ /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

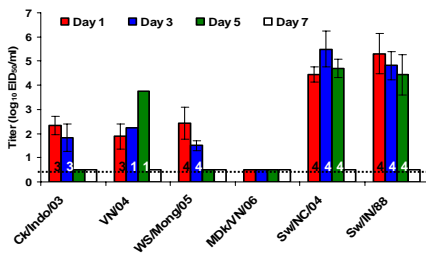
Pig Transmission Model: Experimental Design



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

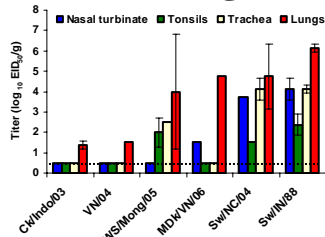
(Lipatov et al., PLOS Pathogens 7:e1000102, 2008)

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)

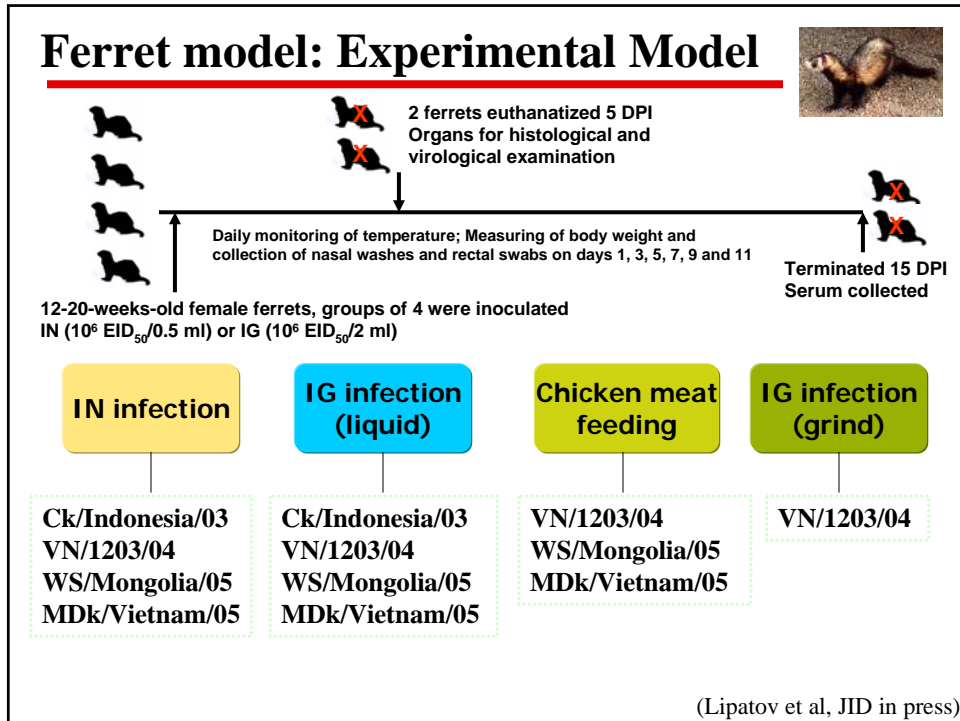
H5N1 HPAIV: IG inoculation

- Liquid media (10^6 EID₅₀ WS/Mong./05 & VN/1203/04), 2 viruses – no evidence of infection
- Feeding WS/05 infected meat (100g, 10^{10} EID₅₀)

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

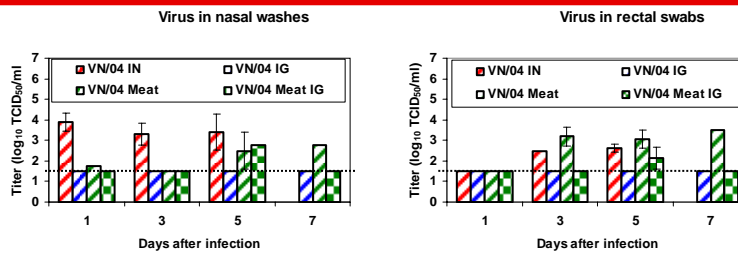
- No lesions
- No weight loss
- No changes in food consumption
- No behavioral abnormalities
- No virus rectal swabs or GI tissues
- Transmission by consumption of infected meat
- Respiratory infection, no evidence of digestive infection

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



- ## Ferret Model: 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. IID 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

GLOBAL PRODUCTION AND TRADE OF POULTRY

Poultry are the most frequently raised farm animals and, on a global basis, birds are the major source of animal protein in the human diet through both meat and eggs. The principal poultry species raised in the chicken, but significant numbers of turkey, duck, guinea, Japanese quail, game bird, and various water species are also raised depending on culture, customs, national production system, and markets. In developed countries, most production and consumption is through specialized integrated commercial farms and chain distribution. In addition, there is a smaller contribution from poultry raised through small colleges, organic, and free poultry market systems that supply some consumers with specialty products such as free or farm-raised birds. In contrast, in many developing countries, the integrated commercial poultry production sectors with cold chain distribution are smaller. The majority of poultry are raised in village or semi-commercial sectors, and free poultry markets supply the local population with poultry meat and eggs.

Global production of chicken meat exceeded 15.8 million metric tons in 2006, of which 97 million metric tons were exported worldwide (30). The top exporting countries, including value added, were Brazil, the United States, the European Union, China, Thailand, Canada, Argentina, the United Arab Emirates, Australia, and Saudi Arabia (30). Smaller quantities of turkey, guinea, duck, and other poultry meat and eggs are also exported each year. The economic importance of poultry production is illustrated by the fact that 12 of the 178 countries report-

ing to the Food and Agriculture Organization (FAO) in 2006 indicated that chickens and eggs were raised within their top five agricultural export categories (2). In 2006, the top 10 exporters of chicken meat were the Russian Federation, Japan, the European Union, Saudi Arabia, Mexico, Ukraine, Hong Kong, China, the United Arab Emirates, and South Africa (30).

In addition to transportation, domestic production and distribution systems are critical for meeting the varying demands of consumers as well as supplying the global market with other products, such as live birds, chicken, poultry and other birds, hatching eggs, and fiber (i.e., feathers). These systems are not only critical for the economic viability of poultry but also contribute to the control of poultry diseases and the prevention of disease spread. This is achieved by implementing sanitary standards and effective disease control programs. Under the Agreement on the Application of Sanitary and Phytosanitary Measures (the so-called SPS Agreement) of the World Trade Organization (WTO), the World Organization of Animal Health (OIE) International Animal Health (IAH) is responsible for establishing science-based standards for sanitary safety in international trade of terrestrial animals and their products including poultry (32). This is achieved by developing and adopting health measures to be used by the countries of origin of exporting and importing countries. The goal of these measures is to prevent the transfer of agents pathogenic for animals or humans while avoiding unjustified trade barriers. The most frequent sanitary restrictions in trade of poultry and poultry products have been related to the

