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Experimental Study to Determine if Low-Pathogenicity and High-Pathogenicity Avian Influenza Viruses Can Be Present in Chicken Breast and Thigh Meat Following Intranasal Virus Inoculation

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SUMMARY. Two low-pathogenicity (LP) and two high-pathogenicity (HP) avian influenza (AI) viruses were inoculated into chickens by the intranasal route to determine the presence of the AI virus in breast and thigh meat as well as any potential role that meat could fill as a transmission vehicle. The LPAI viruses caused localized virus infections in respiratory and gastrointestinal (GI) tracts. Virus was not detected in blood, bone marrow, or breast and thigh meat, and feeding breast and thigh meat from virus-infected birds did not transmit the virus. In contrast to the two LPAI viruses, A/chicken/Pennsylvania/1370/1983 (H5N2) HPAI virus caused respiratory and GI tract infections with systemic spread, and virus was detected in blood, bone marrow, and breast and thigh meat. Feeding breast or thigh meat from HPAI (H5N2) virus-infected chickens to other chickens did not transmit the infection. However, A/chicken/Korea/ES/2003 (H5N1) HPAI virus produced high titers of virus in the breast meat, and feeding breast meat from these infected chickens to other chickens resulted in AI virus infection and death. Usage of either recombinant fowlpox vaccine with H5 AI gene insert or inactivated AI whole-virus vaccines prevented HPAI virus in breast meat. These data indicate that the potential for LPAI virus appearing in meat of infected chickens is negligible, while the potential for having HPAI virus in meat from infected chickens is high, but proper usage of vaccines can prevent HPAI virus from being present in meat.

RESUMEN. Estudio experimental para determinar la presencia de los virus de influenza aviar de baja y alta patogenicidad en carne de pechuga y muslos de pollo, después de la inoculación del virus por vía intranasal.

Se inocularon dos virus de baja patogenicidad y dos virus de alta patogenicidad de influenza aviar en pollos por vía intranasal para determinar la presencia del virus en la carne de pechuga y muslos, y determinar el posible papel potencial de esta carne como vehículo de transmisión. Los virus de influenza aviar de baja patogenicidad ocasionaron infecciones virales localizadas en los tractos respiratorio y gastrointestinal. No se detectaron virus de baja patogenicidad en sangre, médula ósea, carne de pechuga o muslos. No se observó transmisión de los virus a pollos alimentados con carne de pechuga o muslos provenientes de aves infectadas con los virus de influenza aviar de baja patogenicidad. A diferencia de los dos virus de baja patogenicidad, el virus de influenza aviar de alta patogenicidad A/Pollo/Pensilvania/1370/1983/ (H5N2) ocasionó infecciones en los tractos respiratorio y gastrointestinal con difusión sistémica, siendo detectado en sangre, médula ósea y carne de pechuga y muslos. No se observó transmisión del virus a pollos alimentados con carne de pechuga o muslos provenientes de aves infectadas con el virus H5N2 de alta patogenicidad. Sin embargo, el virus de alta patogenicidad A/Pollo/Corea/ES/2003 (H5N1) produjo títulos altos del virus en carne de pechuga y ocasionó infección y muerte en pollos alimentados con carne de pechuga proveniente de aves infectadas por este virus de alta patogenicidad. El uso de vacunas recombinantes de viruela aviar que contienen el gen H5, o de vacunas inactivadas con el virus completo de influenza aviar, previno la presencia del virus de influenza aviar de alta patogenicidad en la carne de pechuga. Los datos indican que el potencial del virus de influenza aviar de baja patogenicidad de encontrarse en carne de pollos infectados es mínima, mientras el potencial de encontrar el virus de alta patogenicidad en carne de pollos infectados es alto, sin embargo, el uso adecuado de vacunas puede prevenir la presencia del virus de alta patogenicidad en la carne de pollo.

Key words: avian influenza, avian influenza virus, high pathogenicity, influenza, low pathogenicity, meat, vaccine

Abbreviations: AAF = amnioallantoic fluid; AGP = agar gel precipitin; AI = avian influenza; BHI = brain heart infusion; EID_{50} = mean embryo infectious dose; GI = gastrointestinal; H = hemagglutinin; H5/HP/83 = A/chicken/Pennsylvania/1370/1983 (H5N2) HPAI virus; H5/HP/03 = A/chicken/Korea/ES/2003 (H5N1) HPAI virus; H7/LP/99 = A/chicken/NY/21586-8/99 (H7N2) LPAI virus; H7/LP/02 = A/turkey/Virginia/158512/2002 (H7N2) HPAI virus; HP = high pathogenicity; LP = low pathogenicity; PI = postinoculation; SPF = specific pathogen free; SEPRL = Southeast Poultry Research Laboratory; WL = White Leghorn; WPR = White Plymouth Rocks

Avian influenza (AI) viruses have differing pathogenesis for the infections and distribution of lesions depending on the virus strain, especially as it relates to pathotypes (16). Experimentally, low-pathogenicity (LP) AI viruses produce infections that are localized to the respiratory and gastrointestinal (GI) tracts of chickens, whereas infections with high-pathogenicity (HP) AI viruses spread systemically, affecting many visceral organs, the brain, and skin (16). In some field outbreaks, LPAI viruses have produced pancreatitis and nephritis, but the mechanism for such infections is unknown.

The World Organization for Animal Health (Office International des Epizooties) establishes animal health standards for safe trade in animals and animal products (6). Currently, two diseases that affect avian species, Newcastle disease and HPAI, have limited distribution in the world, but they produce sufficiently severe disease to be of concern to countries importing poultry and poultry meat. Newcastle disease–free and HPAI-free countries have prohibitions or place restrictions on countries having these diseases. In addition, some importing countries require that exporting countries be free of H5 and H7 LPAI viruses in order for their poultry and poultry products to be exported. The current study was conducted to study the pathogenesis of LPAI and HPAI virus infections in chickens produced by simulated natural exposure (i.e., intranasal inoculation)

and to determine the potential role that breast or thigh meat may serve as a vehicle in transmission of AI viruses.

MATERIALS AND METHODS

Viruses. Four viruses were used in this study: A/turkey/Virginia/158512/2002 (H7N2) LPAI (H7/LP/02), A/chicken/New York/21586-8/99 (H7N2) LPAI (H7/LP/99), A/chicken/Pennsylvania/1370/1983 (H5N2) HPAI (H5/HP/83), and A/chicken/Korea/ES/2003 (H5N1) HPAI (H5/HP/03) viruses. The working stock of the viruses comprised second passage in 10-day-old embryonating chicken eggs via allantoic sac inoculation of the original materials. Amnioallantoic fluid (AAF) was harvested from the eggs at 30–48 hr postinoculation and diluted in brain heart infusion (BHI) medium to obtain a final inoculum titer of approximately 10^6 mean embryo infectious doses (EID_{50}) per chicken (Expts. 1 and 2) and 10^3 EID_{50} per chicken (Expt. 3). Similarly, sterile AAF was diluted in BHI to match the dilution factor of above and was used as the sham inoculum.

Chickens. Three-to-four-week-old White Plymouth Rock (WPR) chickens (Expts. 1 and 2) of mixed sex were obtained from specific-pathogen-free (SPF) stocks maintained at Southeast Poultry Research Laboratory (SEPRL), or we used 4-wk-old SPF White Leghorn (WL) chickens (Expt. 3—feeding on breast and thigh meat) from SEPRL or 1-day-old SPF WL chickens (Expt. 3—vaccine study) from a commercial source (SPAFAS, Storrs, CT). All chickens were housed in negative-pressure, HEPA-filtered, stainless-steel isolation cabinets illuminated under continuous light. Feed and water were provided *ad libitum.*

Experiments 1 and 2: Pathogenesis of AI infection of breast and thigh meat. Individual chickens were intranasally inoculated with approximately 10^6 EID_{50} virus or uninfected sterile allantoic fluid in 0.1 ml into the middle nasal cavity via the choanal cleft. Two experiments to examine presence of AI virus in breast and thigh meat and potential transmission through meat were conducted: 1) Expt. 1—H7/LP/02 virus ($n = 25$) and sham groups ($n = 10$) and 2) Expt. 2—H7/LP/99 ($n = 40$) and H5/HP/83 ($n = 40$) virus and sham ($n = 10$) groups. For Expt. 1, five virus-inoculated chickens were sampled on days 1, 3, 5, 7, and 10 postinoculation (PI). For Expt. 2, five virus-inoculated chickens were sampled on days 1–7 and 10. In both Expts. 1 and 2, five sham-inoculated chickens were sampled on days 3 and 10 PI.

On the day of sampling, blood was collected in EDTA tubes (Vacutainer 366452, Becton Dickinson, Franklin Lakes, NJ) and separated into plasma, erythrocyte, and white-cell fractions (Histopaque, Sigma 1119-1, Sigma Chemical Co., St. Louis, MO). Oropharynx, cloaca, and abdominal air-sac swabs were placed in 1.5 ml of BHI media and stored at −70 C. Lung, trachea, thigh meat, breast meat, and bone (pelvic girdle) were taken and stored at −70 C until virus isolation and titration were performed as previously described (17). For all virus- and sham-inoculated chickens, serum was collected for serologic testing on days 0 and 10 PI, or on day of euthanatization if before day 10 PI.

On day 3 PI, the five chickens per group were processed to collect breast and thigh meat for the AI transmission-feeding test. First, chickens were exsanguinated under anesthesia (xylazine [1.5 mg/kg body weight] and ketamine [15 mg/kg body weight]). Second, thigh (5 g/bird) and breast (10 g/bird) meat samples were collected, pooled, and stored at −70 C. Third, viscera and skin were removed, and the coelom was rinsed with 3 ml of BHI media, which was saved for virus isolation and titration. Fourth, the carcass was immersed in 30-ppm chlorine rinse-water for 60 min at 35–37 F. After chilling, the carcasses were removed and drained, followed by rinsing of the body cavity with 3 ml of media, which was saved for virus isolation and titration.

Thigh and breast meat were fed to chickens to assess transmission potential. Five groups of 10–20 chickens per group were fasted for 12 hr. After 12 hr, finely chopped meat pooled and collected from five chickens on day 3 PI was fed to birds in the following groupings: 1) pooled breast muscle from LPAI virus–inoculated chickens ($n = 20$), 2) pooled thigh muscle from LPAI virus–inoculated chickens ($n = 20$), 3) pooled breast muscle from HPAI virus–inoculated chickens ($n = 10$), 4) pooled thigh muscle from HPAI virus–inoculated chickens ($n = 10$), and 5) pooled breast and thigh muscle from sham-inoculated chickens ($n = 20$). At 14 days postfeeding, the chickens were bled and agar gel precipitin (AGP) serology performed to determine infection rate. The chickens were euthanatized with sodium pentobarbital (100 mg/kg of body weight).

Experiment 3. Effect of vaccination on H5N1 HPAI virus in breast and thigh meat. Groups of 10 one-day-old WL chickens were vaccinated subcutaneously in the nape of the neck with a fowlpox recombinant virus containing hemagglutinin (H) gene of A/turkey/Ireland/83 H5 AI virus (Trovac, Merial, Inc., Gainesville, GA) (15), inactivated A/turkey/Wisconsin/1968 (H5N9) (2.2 μg H protein) emulsified in mineral oil adjuvant (14) or two diluents (both recombinant diluent and noninfected AAF with emulsion). Three weeks after vaccination, sera were collected for serologic testing, and the chickens were inoculated intranasally with $10^{3.3}$ EID_{50} of H5/HP/03 AI virus. On day 2 PI, three birds were euthanatized from fowlpox-AIV-H5 recombinant and inactivated AI vaccine groups, and breast and thigh meat were collected and virus reisolation and titration performed. Breast meat from nine diluent-vaccinated chickens was collected on day of death and virus reisolation and titration performed. From the diluent vaccine group, 16.75 g of pooled breast meat was fed directly to five 12-hr fasted 4-wk-old WL chickens and 16.75 g of pooled breast meat was fed in water to five 12-hr fasted 4-wk-old WL chickens. The chickens were observed for mortality, and virus isolation was performed on oropharyngeal and cloacal swabs.

Serology. Serum was tested for type A influenza antibodies using the AGP test and hemagglutination inhibition test for H5 subtype (17).

RESULTS

Experiments 1 and 2. Pathogenesis of intranasal infections. The data sets from the two sham groups were identical and were pooled for all evaluations (Tables 1, 2). All the chickens sampled in the three virus-inoculated groups became infected, as demonstrated by either virus isolation from oropharyngeal swabs or seroconversion (Table 1).

LPAI groups. For the two LP virus–inoculated groups, virus replication occurred in the respiratory and GI tracts, with the highest average titers for each time point being those for the H7/LP/99 group (Tables 1, 2). Virus replication was first evident on day 1 PI in the upper respiratory tract, with the last positive sample either on day 5 (H7/LP/02) or day 7 (H7/LP/99) PI and the average peak replication ($10^{4.2}$ and $10^{5.5}$ EID_{50}/ml, respectively) on day 3 PI (Table 1). Replication was detected in the lower respiratory tract (trachea and lung) on day 3 PI, with the last positive for virus replication on day 5 (H7/LP/02) or day 7 (H7/LP/99) PI. Average peak replication was on day 3 PI (Table 2). Virus was rarely detected in the deep respiratory tract (air sacs) of chickens in the H7/LP/99 group (8%) and was not detected in chickens from H7/LP/02 group (Table 1). From the GI tract (cloacal swab), virus was rarely detected in the H7/LP/02 group (4%), but with the H7/LP/99 group, virus was detected commonly (43%), days 2–7 PI, and had the highest average peak titer on day 4 PI ($10^{4.3}$ EID_{50}/ml) (Table 1). Avian influenza virus was not isolated from the blood fractions or bone or breast and thigh meat samples (Table 2).

No virus was recovered from pre- and postchill internal carcass rinses of sham or H7/LP/02 groups (Table 3). Pre- and postchill internal carcass rinses were AI virus positive from two of five H7/LP/99-inoculated chickens, and these chickens had virus-positive tracheal and lung tissues (Table 3). The average AI virus titers were $10^{5.4}$ and $10^{3.9}$ EID_{50}/ml for pre- and postchill rinses, respectively (Table 3).

HPAI group. The H5/HP/83 group had virus replication in respiratory and GI tracts and internal tissues. Virus was isolated from

the upper and lower respiratory tracts (oropharyngeal swabs, and trachea and lung) beginning on day 1 PI and persisted through day 5 PI, with all groups having high average peak titers on day 3 ($10^{5.0–6.5}$ EID_{50}/g or ml) (Tables 1, 2). Coinciding with respiratory infection, a viremia was detected in white and red blood cell fractions at day 1 PI and continued through day 3 PI (Table 2). Virus was uncommon in the plasma fraction (7%) (Table 2). Infection was present in bone marrow and breast and thigh meat on days 1, 3, and 5, but the titers were lower than in the trachea or lung (Table 2). The deep respiratory tract (air sac) had virus on days 3 and 5 (Table 1). Because all remaining birds died on day 5 PI, no chickens were available for sampling on days 7 or 10 PI. These chickens had lesions of HPAI including petechial hemorrhages on viscera, hemorrhage and necrosis of comb and wattles, and edema of feet. All pre- and postchill rinses of coelom contained AI virus, with average titers of $10^{3.0}$ and $10^{3.3}$ EID_{50}/ml, respectively (Table 3).

Experiments 1 and 2. Transmission of infection via feeding breast and thigh meat. No AI virus was isolated from the breast and thigh meat obtained from chickens in the sham, H7/LP/02, or H7/LP/99 groups, but breast and thigh meat obtained from chickens in the H5/HP/83 group had average titers of $10^{2.2–2.7}$ and $10^{2.6–3.2}$ EID_{50} virus/g of breast and thigh meat, respectively, between days 1 and 5 PI (Table 4). To determine if meat can be a transmission vehicle for AI, breast and thigh meat from the sham and three virus-inoculated birds obtained on day 3 PI was fed to 12-hr fasted chickens. All the meat was consumed within 5 min. No AI virus was isolated from breast and thigh meat obtained from chickens in the sham or LPAI groups. None of the chickens fed the breast and thigh meat from sham or virus-inoculated groups died, developed clinical signs, or produced antibodies to AI virus. Breast and thigh meat obtained from H5/HP/83 AI virus–infected chickens contained $10^{2.7}$ and $10^{3.2}$ EID_{50} of virus/g, and SPF chickens fed this meat received an average $10^{3.5}$ and $10^{3.6}$ EID_{50} of virus per chicken, respectively (Table 4).

Experiment 3. Effect of vaccination on H5N1 HPAI virus in breast and thigh meat. At three wk postvaccination, chickens in fowlpox-AIV-H5 recombinant and inactivated AI vaccine groups were positive for antibodies to H5 AI H, and the inactivated AI vaccine group had antibodies to influenza virus type A group-specific antigens (AGP) (Table 5). However, chickens in the diluent group were AI virus antibody negative (Table 5). After H5/HP/03 challenge, high titers of AI virus were isolated from breast meat of chickens in the diluent group that died (average $10^{7.3}$ EID_{50} virus/g), but virus was not isolated from breast or thigh meat from chickens of either vaccine groups euthanatized on day 2 PI (Table 5). Feeding breast meat from the H5/HP/03 AI virus-challenged diluent group (mean dose of $10^{7.8}$ EID_{50} of virus per chicken) to 10 SPF WL chickens resulted in death on day 2 after feeding; four of five chickens that consumed AI-infected breast meat (16.75 total g of meat) died. Five of five chickens that consumed the AI-infected breast meat in drinking water (16.75 total g of meat) died. All nine chickens that died had isolation of H5 HPAI virus from oropharyngeal and cloacal swabs. The surviving chicken from the group fed the breast meat directly did not seroconvert to the AI virus.

Table 1. Serologic[A] and respiratory virologic data for 3-to-4-wk-old chickens inoculated with sterile allantoic fluid (sham) or AI viruses (H7/LP/02, H7/LP/99, and H5/HP/83).

Group	Day of sample	Serology (AGP)	Virus isolation from swabs (log_{10} EID_{50}/ml)[B]		
			Oropharyngeal	Cloacal	Air sac
Sham	3	0/10	0/10	0/10	0/10
	10	0/10	0/10	0/10	0/10
	Subtotal	0/20	0/20	0/20	0/20
H7/LP/02	1	0/5	5/5 (3.6)	0/5	0/5
	3	0/5	5/5 (4.2)	0/5	0/5
	5	2/5	5/5 (3.3)	1/5 (1.0)	0/5
	7	5/5	0/5	0/5	0/5
	10	5/5	0/5	0/5	0/5
	Subtotal	12/25	15/25	1/25	0/25
H7/LP/99	1	0/5	5/5 (4.1)	0/5	0/5
	2	NS[C]	5/5 (3.5)	1/5 (1.5)	NS
	3	0/5	5/5 (5.5)	2/5 (2.2)	0/5
	4	NS	5/5 (5.1)	4/5 (4.3)	NS
	5	2/5	5/5(4.4)	4/5 (3.4)	2/5 (2.8)
	6	NS	5/5 (2.5)	3/5 (3.0)	NS
	7	5/5	3/5 (1.1)	3/5 (1.5)	0/5
	10	5/5	0/5	0/5	0/5
	Subtotal	12/25	33/40	17/40	2/25
H5/HP/83	1	0/5	5/5 (5.8)	1/5 (1.0)	0/5
	2	NS	5/5 (5.5)	5/5 (4.5)	NS
	3	0/5	5/5 (6.5)	5/5 (2.6)	3/5 (2.0)
	4	NS	5/5 (6.5)	4/5 (2.3)	NS
	5	5/5	5/5 (6.1)	4/5 (1.9)	4/5 (2.5)
	Subtotal	5/15	25/25	19/25	7/15

[A]All birds ($n = 85$) were negative for antibodies against avian influenza virus on the day of challenge (day 0 PI).
[B]Minimum detection limit of $10^{0.9}$ EID_{50}/ml.
[C]No sample.

DISCUSSION

The production of influenza virus infection requires multiple replication cycles of the virus in the host animal and necessitates a prerequisite for intra- or extracellular cleavage of the surface H protein (HA0) into two subunits, HA1 and HA2, to produce these multiple replication cycles (16). Cleavage of the HA0 to HA1 and HA2 exposes regions that allow binding and fusion of the virus to specific receptors on host cells and initiation of the virus replication process. The type of cells that are permissive for AI virus replication in chickens are restricted by the host cytoplasmic enzymatic machinery and the proteolytic cleavage site structure of the H of the AI virus. The HA0 of LPAI viruses is cleaved by trypsin-like enzymes, which are predominantly present in epithelial cells, especially in respiratory and GI tracts. By contrast, the HA0 of the HPAI viruses is cleaved by the furin class of ubiquitous proteases found in most cells throughout the body (10). As a result, LPAI viruses produce localized virus infection in respiratory and GI tracts, whereas the HPAI viruses begin as infections of respiratory and GI tracts, which spread systemically and replicate in multiple internal organs.

In the current study, the pathogenesis of the LP and HPAI virus infections in chickens presented dramatic differences in tissue tropism and transmission of virus through the breast and thigh meat. Intranasal inoculation of broiler type chickens (WPR) with two different LPAI viruses resulted in predominant infection in the upper and lower respiratory tract between days 1 and 7 PI, with the peak in virus replication occurring at day 3 PI. However, infection in the GI tract varied between the two LPAI viruses; the H7/LP/02 LPAI virus had poor replication in the GI tract (4% AI virus–positive cloacal swabs), whereas H7/LP/99 LPAI virus had a strong predilection (43% AI virus–positive cloacal swabs) for replication in the GI tract. Both the LPAI viruses failed to produce viremias, and no virus was isolated from bone or breast and thigh meat. Previous studies with LPAI viruses in chickens failed to demonstrate virus in skeletal muscle, but in some reports, AI viruses were detected in or isolated from lungs and air sacs (4,9) (Rosenberger, pers. comm.). In the current study, the detection of LPAI virus in coelomic rinses,

Table 2. Serologic and respiratory virologic data for 3-to-4-wk-old chickens inoculated with sterile allantoic fluid (sham) or AI viruses (H7/LP/02, H7/LP/99, and H5/HP/83).

Group	Day sampled	Blood (log_{10} EID_{50}/ml)[A]			Tissues (log_{10} EID_{50}/g)[B]				
		Plasma	WBC	RBC	Trachea	Lung	Bone	Breast meat	Thigh meat
Sham	3	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	Subtotal	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
H7/LP/02	1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	3	0/5	0/5	0/5	3/5 (3.8)	3/5 (3.5)	0/5	0/5	0/5
	5	0/5	0/5	0/5	3/5 (3.7)	3/5 (2.2)	0/5	0/5	0/5
	7	0/5	0/5	0/5	1/5 (2.0)	1/5 (2.2)	0/5	0/5	0/5
	10	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	Subtotal	0/25	0/25	0/25	7/25	7/25	0/25	0/25	0/25
H7/LP/99	1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	3	0/5	0/5	0/5	2/5 (5.5)	2/5 (4.6)	0/5	0/5	0/5
	5	0/5	0/5	0/5	3/5 (3.5)	3/5 (3.2)	0/5	0/5	0/5
	7	0/5	0/5	0/5	0/5	1/5 (2.9)	0/5	0/5	0/5
	10	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	Subtotal	0/25	0/25	0/25	5/25	6/25	0/25	0/25	0/25
H5/HP/83	1	0/5	4/5 (1.2)	5/5 (1.4)	3/5 (2.8)	5/5 (3.4)	4/5 (2.3)	2/5 (2.2)	3/5 (2.6)
	3	1/5 (1.5)	3/5 (1.2)	4/5 (1.4)	5/5 (5.0)	5/5 (6.0)	5/5 (3.0)	5/5 (2.7)	5/5 (3.2)
	5	0/5	0/5	0/5	5/5 (4.1)	5/5 (5.5)	5/5 (2.7)	4/5 (2.5)	5/5 (2.6)
	Subtotal	1/15	7/15	9/15	13/15	15/15	14/15	11/15	13/15

[A]Minimum detection limit of $10^{0.9}$ EID_{50}/ml.
[B]Minimum detection limit of $10^{1.9}$ EID_{50}/g.

both pre- and postchill, indicates that residual virus may have remained in the thoracic region after lung removal. Other natural infections or intranasal experimental studies in chickens have shown some LPAI viruses have caused lesions in the kidneys and oviducts, especially in reproductively active hens (1,23). It is unclear if such lesions were associated with LPAI virus replication in these visceral organs or if the lesions resulted from secondary pathophysiologic events or other agents without LPAI virus. In one study, virus was isolated in kidney samples obtained from hens intranasally inoculated with A/chicken/Alabama/75 (H4N6) LPAI virus, but the virus was localized by immunohistochemistry to epithelium of the overlying abdominal air sac and not to the parenchyma of the kidney (9). Other studies with intravenous inoculation of LPAI viruses in chickens have demonstrated replication in kidneys, but not meat (13,18,20). In contrast to this aberrant route of exposure (i.e., intravenous inoculation), intranasal inoculation studies in chickens have not identified LPAI virus in parenchymal cells of kidneys or skeletal muscle fibers (11,19).

By contrast, in the current studies, the H5/HP/83 HPAI virus replicated in upper, lower, and deep portions of the respiratory tract of chickens with higher peak replication titers in oropharyngeal swabs and lungs than were seen with LPAI viruses. In addition, the HPAI virus sustained high titers longer in the trachea than did LPAI viruses, and the HPAI virus had an earlier peak titer and more positive samples in GI tract than LPAI viruses. In the current and previous studies, HPAI virus infections produced viremia and systemic infection with the virus being present in the whole body of the chicken (3,5). HPAI viruses have been isolated from or demonstrated in skeletal muscle of experimentally infected chickens (2,4,5,7,8) (Rosenberger, pers. comm.). Previously, an H5N1 HPAI virus was isolated from imported duck carcasses in South Korea. In intranasal experimental inoculation, this virus was isolated from and demonstrated in breast and thigh meat of chickens with severe clinical signs and in thigh meat from clinically normal ducks (21,22).

In the current study, the level of virus detected in skeletal muscle varied with HPAI virus strain; i.e., H5/HP/83 and H5/HP/03 had $10^{2.7–3.2}$ and $10^{7.3}$ EID_{50}/g of breast or thigh meat, respectively. Feeding H5/HP/83 AI virus–infected chicken breast or thigh meat to SPF chickens ($10^{3.5–3.6}$ EID_{50}/bird) did not produce infection, but

Table 3. Virologic data from pre- and postchill body cavity rinses from 3-to-4-wk-old chickens inoculated with sterile allantoic fluid (sham) or AI viruses (H7/LP/02, H7/LP/99, and H5/HP/83).

Group	Virus isolation (log_{10} EID_{50}/ml)	
	Prechill	Postchill
Sham	0/10[A]	0/10
H7/LP/02	0/5	0/5
H7/LP/99	2/5 (5.4)	2/5 (3.9)
H5/HP/83	5/5 (3.0)	5/5 (3.3)

[A]Number positive/total tested.

Table 4. Data from Expts. 1 and 2 for breast and thigh meat collected on day 3 PI from 3-to-4-wk-old chickens inoculated with sterile allantoic fluid (sham) or AI viruses (H7/LP/02, H7/LP/99, and H5/HP/83) (provider) and whose meat was fed to 3-to-4-wk-old chickens (recipient) to assess virus transmission.

	Provider chickens			Recipient chickens	
Group	Type	Quantity (g)	Titer (log_{10} EID_{50}/g)	Average virus dose/bird (log_{10} EID_{50})	Serology day 14 postfeeding (AGP)
Sham	Breast, thigh	112.2, 59.1	—[A]	—	0/20[B]
H7/LP/02	Breast	57.2	—	—	0/10
	Thigh	27.4	—	—	0/10
H7/LP/99	Breast	54.2	—	—	0/10
	Thigh	28.0	—	—	0/10
H5/HP/83	Breast	58.1	2.7	3.5	0/10
	Thigh	26.7	3.2	3.6	0/10

[A]— = No virus was isolated from the meat samples.
[B]Number positive/total tested.

Table 5. Data from Expt. 3 for breast and thigh meat collected on day 2 PI from 3-to-4-wk-old chickens inoculated with H5/HP/03 HPAI virus (provider) and fed to 3-to-4-wk-old chickens (recipient) to assess virus transmission.

	Provider chickens					
	Serology		Virus isolation from meat (log_{10} EID_{50}/g)		Recipient chickens	
Group	AGP	HI titer (GMT)	Breast	Thigh	Virus dose/bird (log_{10} EID_{50})	Mortality
Fowlpox-AIV-H5 vaccine	0/3[A]	3/3 (315)	—[B]	—	ND[C]	ND
Inactivated vaccine	3/3	3/3 (79)	—	—	ND	ND
Diluent	0/10	0/10	7.3	ND	7.8	9/10

[A]Number positive/total tested.
[B]— = Negative on virus isolation.
[C]ND = not done.

90% of chickens fed breast meat from H5/HP/03 HPAI virus–infected chickens ($10^{7.8}$ EID_{50}/bird) became infected and died. This difference in transmission by feeding HPAI virus–infected breast or thigh meat could be a dose effect because the mean chicken infectious dose for the H5/HP/83 and H5/HP/03 HPAI viruses were similar (10^{3} and $10^{2.45}$ EID_{50}, respectively; unpubl. data), but the virus exposure dose was 10^{4} EID_{50} higher for the H5/HP/03 HPAI virus than for the H5/HP/83 HPAI virus. Alternatively, lack of direct exposure to the respiratory tract (i.e., oral feeding of meat) may have required a higher exposure dose to achieve infection, thus explaining why there was infection only with H5/HP/03 HPAI virus and not with H5/HP/83 HPAI virus. In a previous study, feeding of 5 g of meat from HPAI virus–infected chickens did not transmit HPAI to other chickens, but feeding of 0.5 g of blood from infected chickens did transmit HPAI (8). The quantity of breast or thigh meat fed in the current study was similar at 2.7–5.8 g/chicken.

Properly used vaccines are effective at preventing clinical signs and death (12). In addition, vaccines can decrease the number of birds with AI virus replication and can reduce the quantity of virus replication in respiratory and GI tracts (12). In the current study and another study (2), vaccination with either fowlpox-AIV-H5 recombinant or inactivated AI virus vaccines prevented H5/HP/03 and A/turkey/Italy/4580/V99 (H7N1) HPAI virus replication in breast meat. This indicates that vaccination can be used as a tool to prevent HPAI virus replication in skeletal muscle and thus minimize its potential as a vehicle for transmission of HPAI virus.

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