Thermal Inactivation of Avian Influenza and Newcastle Disease Viruses in Chicken Meat

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ABSTRACT

Avian influenza viruses (AIV) and Newcastle disease viruses (NDV) of high pathogenicity cause severe systemic disease with high mortality in chickens and can be isolated from the meat of infected chickens. Although AIV and NDV strains of low pathogenicity are typically not present in chicken meat, virus particles in respiratory secretions or feces are possible sources of carcass contamination. Because spread of AIV and NDV is associated with movement of infected birds or their products, the presence of these viruses in chicken meat is cause for concern. This study presents thermal inactivation data for two viruses of high pathogenicity in chickens (AIV strain A/chicken/Pennsylvania/1370/1983 and NDV strain APMV-1/chicken/California/S0212676/2002) and two viruses of low pathogenicity in chickens (AIV strain A/chicken/Texas/298313/2004 and NDV strain APMV-1/chicken/Northern Ireland/Ulster/1967). Under the conditions of the assay, high-pathogenicity AIV was inactivated more slowly in meats from naturally infected chickens than in artificially infected meat with a similar virus titer. In contrast, high-pathogenicity NDV was inactivated similarly in naturally and artificially infected meat. Linear regression models predicted that the current U.S. Department of Agriculture—Food Safety and Inspection Service time-temperature guidelines for cooking chicken meat to achieve a 7-log reduction of Salmonella also would effectively inactivate the AIV and NDV strains tested. Experimentally, the AIV and NDV strains used in this study (and the previously studied H5N1 high-pathogenicity AIV strain A/chicken/Korea/ES/2003) were effectively inactivated in chicken meat held at 70 or 73.9°C for less than 1 s.

Avian influenza virus (AIV) and Newcastle disease virus (NDV) strains cause disease in many species of domestic and wild birds. Differentiation of AIV strains is primarily by classification into 16 hemagglutinin (H) and 9 neuraminidase (N) subtypes (7). All NDV strains are of a single type, avian paramyxovirus type 1 (APMV-1) (1). The severity of disease depends on multiple factors, such as viral virulence, host susceptibility, and environmental conditions (1, 19). For both AIV and NDV, cleavage of a surface protein precursor (hemagglutinin precursor in AIV and fusion protein precursor in NDV) by host cell proteases is required for viral infectivity. Therefore, recognition of the cleavage site by host cell proteases is a critical determinant of pathogenicity and tissue tropism for AIV and NDV strains and their classification as either low or high pathogenicity and low or high virulence, respectively. The terms low pathogenicity (LP) and high pathogenicity (HP) are used herein for both AIV and NDV. In chickens, replication of LP AIV and LP NDV strains is typically restricted to the respiratory and/or digestive tract, and infections usually are asymptomatic or mild in the absence of coinfecting pathogens. In contrast, HP AIV and HP NDV strains have hemagglutinin (AIV) or fusion protein (NDV) cleavage sites recognized by host cell proteases in a wide variety of cell types. These strains can cause severe systemic disease with high mortality in chickens. The virus is present in the blood (viremia) and can be isolated from multiple tissues. In numerous studies, HP AIV has been reported in meat from infected chickens (17, 18, 22, 23), and HP AIV antigen has been detected in skeletal muscle cells (11, 13, 23). Although detection of HP NDV antigen in skeletal muscle cells of infected chickens has not been reported, HP NDV is present in the blood and therefore possibly may be isolated from the meat of infected chickens (3).

AIV and NDV can be spread through the movement of diseased poultry, contaminated poultry products, or contaminated equipment and supplies. The spread of AIV and NDV through trade activity is of concern because of the high economic cost of controlling these diseases in poultry and, in the case of human infective AIV strains, the possibility of transmission to humans. The World Organization for Animal Health (OIE), the intergovernmental organization that establishes health standards for international trade in animals and animal products, recommends that poultry products from HP AIV– or HP NDV–infected countries, zones, or compartments be processed to ensure the destruction of virus prior to export (12). Because HP AIV strains can evolve from LP AIV precursors of the hemagglutinin subtypes H5 and H7 (5), the OIE considers H5 and H7 AIV strains that cause LP infections in chickens but have hemagglutinin precursor cleavage sites similar to those found in HP AIV isolates to be HP AIV for international trade purposes (12).

AIV and NDV are relatively heat-labile enveloped RNA viruses. Therefore, thermal processing could be a
good method for inactivating these viruses in poultry products. Thermal inactivation studies have been conducted with HP AIV in meat from infected chickens (17, 22), HP AIV in artificially infected chicken meat suspension (8), and HP NDV in artificially infected chicken meat homogenate (2). In the current study, we present thermal inactivation data for two H5N2 AIV strains (one HP and one clinically LP in chickens but with a hemagglutinin precursor protein cleavage site similar to that found in HP NDV isolates) and two NDV strains (one HP and one LP) in chicken meat. Mathematical models were used to predict the log reduction in AIV and NDV expected in raw, skinless chicken meat cooked according to current U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) guidelines, and predictions for AIV and NDV inactivation at 70 and 73.9°C were tested experimentally.

MATERIALS AND METHODS

Viruses. The following AIV strains were used in this study: A/chicken/Korea/ES/2003 (Korea03) (H5N1), A/chicken/Pennsylvania/1370/1983 (PA/83) (H5N2), and A/chicken/Texas/298313/2004 (TX/04) (H5N2). The following NDV strains were used in this study: APMV-1/chicken/California/S0212676/2002 (CA/02) and APMV-1/chicken/Northern Ireland/Ulster/1967 (Ulster). Because AIV Korea03, AIV PA/83, and NDV CA/02 are HP avian viruses and AIV TX/04 is treated as such for international trade purposes, work with these viruses and infected material was performed in USDA-certified biosafety level 3 agriculture (BSL-3Ag) facilities. Work with the LP NDV Ulster virus and meat artificially infected with NDV Ulster was performed in BSL-2 facilities. Working stocks of AIV and NDV were grown in 10-day-old embryonating specific-pathogen-free (SPF) chicken eggs. Amnioallantoic fluid was harvested 2 days (AIV strains and NDV CA/02) or 4 days (NDV Ulster) after allantoic sac inoculation. To prepare inocula for animal experiments, an aliquot from each working stock was diluted in protein-rich buffered medium Bacto brain heart infusion (BHI; Becton Dickinson, Sparks, Md.) to deliver a mean embryo infective dose (EID_{50}) of approximately 6 log EID_{50} per bird in 0.1 ml.

Animal experimental design. All experiments with infected animals and material from infected animals were performed in BSL-3Ag facilities. Chickens were housed in negative-pressure high-efficiency particulate air–ventilated stainless steel isolation cabinets under constant illumination. Feed and water were provided ad libitum. For each virus, five 4-week-old White Leghorn SPF chickens were each inoculated intranasally, which simulates natural exposure to these viruses. For PA/83, a second group of five White Leghorn SPF chickens of a different age (5 weeks old) were each inoculated following the same procedures. All of the chickens infected with AIV PA/83 or NDV CA/02 died or were euthanized because of severe illness at 3 to 5 days postinfection. None of the chickens infected with AIV TX/04 or NDV Ulster displayed clinical signs, and chickens in both groups were euthanized at 3 days postinfection. Meat and oropharyngeal and cloacal swab samples were taken from each chicken after death or euthanasia. Swabs were placed in 1.5 ml of BHI containing appropriate antibiotics (20), and thigh and breast meat samples were stored at −70°C. Meat samples from a minimum of two chickens with HP infections or three chickens with LP infections were processed for virus isolation and titration.

Meat samples. Raw skinless meat samples (0.05 ± 0.002 g) were dispensed into thin-walled PCR tubes and centrifuged to pack the meat into the bottom of the tubes. No virus was added to meat from infected chickens. Artificially infected meat samples were prepared by injecting 1 μl of virus stock into the center of 0.05 g of breast meat from uninfected SPF chickens. After heat treatment and chilling, meat samples were processed as described previously (17, 22). Meat samples were ground in microfuge tubes with small pestles, and 0.5 ml of BHI containing appropriate antibiotics was added to each ground meat sample to form a 10% tissue suspension. The tissue suspensions were clarified by centrifugation, and the supernatants were kept for virus isolation and titration.

Liquid samples. For thermal inactivation experiments in liquid medium, stock virus titer was adjusted to approximately 8.0 log EID_{50}/ml by the addition of sterile amnioallantoic fluid from uninfected SPF chicken eggs, and 0.1-ml aliquots were dispensed into thin-walled PCR tubes.

Thermal inactivation procedure. Thermal inactivation experiments were performed as described previously (22). Triplicate samples were prepared for most time points, but in some cases additional samples were tested (duplicate run). Samples were placed in the thermocycler heating block at 25°C for the specified length of time at the target temperature and then chilled to 4°C. For the zero time point at each temperature, samples were removed immediately after the heating block reached the target temperature.

Virus isolation and titration. Dilution series were prepared in BHI, and 0.1 ml of each dilution was inoculated into each of three 9- to 11-day-old embryonating chicken eggs (20). The 50% endpoints were calculated using the method of Reed and Muench (25). The detection limit of the assay was 2.2 log EID_{50}/g for meat and 1.2 log EID_{50}/ml for liquid samples. Swab samples were assayed for virus by inoculating 0.2 ml of undiluted sample into each of three eggs as described above (the detection limit was 0.9 log EID_{50}/ml).

Statistics and graphs. Statistical operations were performed with Sigma Stat version 2.03 (1992 through 1997, SPSS, Chicago, Ill.). Graphs were prepared with Sigma Plot (2000, SPSS). The distribution of the virus titer data was approximately lognormal (the mean was approximately equal to the median) and fulfilled the normality requirement for parametric statistical tests. Experimental D-values were calculated from the linear regression of virus titer versus time at the given temperature (D = −1/slope), and z-values and regression line equations for D-value prediction were obtained from linear regression plots of D-values (log scale) versus temperature (z = −1/slope). The following equation was used to calculate the upper limits of the 95% prediction intervals for the D-values:

$$y + 2\times \text{RMSE}$$

where y is the predicted log D-value (seconds) and RMSE is the root mean square error (standard error of the y estimate).

RESULTS

Amount of virus present in meat from infected chickens. Chickens were inoculated intranasally, which simulates natural respiratory exposure to these viruses. AIV PA/83 and NDV CA/02 caused severe systemic infections, and infectious virus was recovered from meat samples. Average NDV CA/02 titers were 6.8 log EID_{50}/g in thigh meat.
and 6.4 log EID₅₀/g in breast meat. AIV PA/83 titers were determined for only breast meat and averaged 6.7 log EID₅₀/g in 4-week-old chickens and 4.9 log EID₅₀/g in 5-week-old chickens. In contrast, birds infected with AIV TX/04 and NDV Ulster displayed no clinical signs, and infectious virus was not detected in meat samples. However, virus isolation from oropharyngeal swab samples confirmed that the asymptomatic birds were infected. The pathogenicity of these AIV and NDV strains in chickens was as expected, based on published information (1, 10, 18, 26). Although AIV TX/04 is LP based on in vivo chicken pathotyping tests, this strain is considered HP by the OIE for international trade purposes because it has a hemagglutinin precursor protein cleavage site similar to that found in HP AIV isolates (10).

Heat inactivation of AIV and NDV in meat from infected chickens versus artificially infected meat samples. Because AIV TX/04 and NDV Ulster were not detected in meat from infected chickens, thermal inactivation experiments with these strains were performed in artificially infected meat samples. AIV TX/04 inactivation was rapid in artificially infected meat samples at the relatively low temperature of 51°C, and all detectable virus was inactivated during the 27 s that it took the thermocycler block to heat from 25 to 57°C (Table 1). These results were unexpected because such rapid inactivation of AIV in chicken meat at 57°C was not observed in a previous study with the H5N1 HP AIV Korea/03 strain (22). Unlike AIV TX/04, the AIV Korea/03 strain causes systemic infections in chickens. For the AIV Korea/03 inactivation study, meat from infected chickens was used rather than artificially infected meat. Therefore, rapid AIV TX/04 inactivation could be due to one or more of the following: increased heat sensitivity of AIV in artificially infected meat versus meat from AIV-infected chickens, the approximately 1-log

**Table 1: Thermal inactivation of AIV TX/04 in artificially infected chicken meat**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>No. of positive samples</th>
<th>Avg titer after treatment (log EID₅₀/g)</th>
<th>No. of positive samples</th>
<th>Avg titer after treatment (log EID₅₀/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
<td>3.7</td>
<td>0</td>
<td>≤2.1</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>2.4</td>
<td>0</td>
<td>≤2.1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>≤2.2</td>
<td>0</td>
<td>≤2.1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>≤2.5</td>
<td>0</td>
<td>≤2.1</td>
</tr>
<tr>
<td>4</td>
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<td>0</td>
<td>≤2.1</td>
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<td>5</td>
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<tr>
<td>6</td>
<td>0</td>
<td>≤2.1</td>
<td>0</td>
<td>≤2.1</td>
</tr>
</tbody>
</table>

EID₅₀/g, mean embryo infective dose. The average titer of untreated meat samples was 6.7 log EID₅₀/g.

For 0-min treatments, samples were removed immediately after the heating block reached the target temperature.

Three samples were tested at each time point.

Detection limit of the assay was 2.2 log EID₅₀/g. Negative samples were assigned the value ≤2.1 log EID₅₀/g for calculating average titers.

To determine which factor(s) contributed to rapid heat inactivation of AIV TX/04, heat inactivation of the HP AIV PA/83 in artificially infected meat samples was compared to that in meat harvested from two different AIV PA/83-infected chickens (with different AIV PA/83 titers). AIV PA/83 was not detected in artificially infected meat after heating to 57°C but could still be isolated from the meat of both AIV PA/83-infected chickens after 6 min at 57°C (Fig. 1A). These results indicate that the rapid AIV inactivation observed in the artificially infected meat was primarily due to the infection method rather than the AIV strain or the virus titer. Further support for this conclusion was obtained by comparing heat inactivation of three different AIV strains (PA/83, TX/04, and Korea/03) at similar concentrations in similar environments (liquid medium). The survival curves and D-values (time required for a 1-log reduction in virus titer) obtained for each of the three AIV strains in chicken egg allantoic fluid at 57°C were not dramatically different (Fig. 1B).

To determine whether technical problems were responsible for the rapid AIV inactivation in artificially infected meat samples, inactivation of a different virus (HP NDV CA/02) was compared in meat from infected chickens versus artificially infected chicken meat. Similar survival curves were obtained for NDV CA/02 in meat from NDV CA/02-infected chickens and in artificially infected meat samples at 59°C, and the D-values obtained from the survival curves differed by only 1% (Fig. 1C). These results suggest that the difference observed in this study for AIV inactivation in meat from AIV-infected chickens versus artificially infected meat was specific for AIV.

**Survival curves for AIV and NDV in chicken meat.**

Figure 2 shows survival curves for AIV PA/83, NDV CA/02, and NDV Ulster in chicken meat at temperatures ranging from 57 to 61°C at 1°C intervals. The AIV PA/83 and NDV CA/02 experiments were done with meat from infected chickens, and the NDV Ulster experiments were done with artificially infected meat. Because in a previous study AIV Korea/03 inactivation was not significantly different in breast versus thigh meat (22), experiments with AIV PA/83 were done in only breast meat. Similar survival curves were obtained for NDV CA/02 in breast versus thigh meat at 57, 59, and 61°C (Fig. 2). Therefore, experiments with NDV CA/02 at 58 and 60°C were done with only thigh meat. NDV Ulster experiments were done with only breast meat.

The coefficients of determination (R²) and the D-values calculated from each survival curve are listed in Table 2. Although some of the survival curves were slightly biphasic, a linear model provided a fair-to-good fit for most of the curves. R² values ranged from 0.84 to 0.95 for all curves except those for NDV Ulster at 57 and 59°C. The shape of the 59°C curve for NDV Ulster probably was an experimental artifact; the curves for 58 and 60°C were more linear. For calculating D-values, a linear model was as-

**Table 2: Survival curves for AIV and NDV in chicken meat**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AIV PA/83</th>
<th>NDV CA/02</th>
<th>NDV Ulster</th>
</tr>
</thead>
<tbody>
<tr>
<td>57°C</td>
<td>0.95</td>
<td>0.95</td>
<td>0.95</td>
</tr>
<tr>
<td>59°C</td>
<td>0.92</td>
<td>0.92</td>
<td>0.92</td>
</tr>
<tr>
<td>61°C</td>
<td>0.91</td>
<td>0.91</td>
<td>0.91</td>
</tr>
</tbody>
</table>

The coefficients of determination (R²) and the D-values calculated from each survival curve are listed in Table 2. Although some of the survival curves were slightly biphasic, a linear model provided a fair-to-good fit for most of the curves. R² values ranged from 0.84 to 0.95 for all curves except those for NDV Ulster at 57 and 59°C. The shape of the 59°C curve for NDV Ulster probably was an experimental artifact; the curves for 58 and 60°C were more linear. For calculating D-values, a linear model was as-
sumed for all curves. However, a nonlinear model would probably describe the data for the 57 and 59°C NDV Ulster curves with less error.

Attempts to construct accurate survival curves for AIV TX/04 in artificially infected meat at lower temperatures were not successful because of the low levels of virus remaining after the meat was heated to the target temperature. The data shown in Figure 1A and Table 1 suggest that cooking chicken meat to inactivate systemic AIV strains would also inactivate AIV from other sources (such as respiratory or intestinal secretions) that could contaminate meat during the slaughter and evisceration processes. The data shown in Figure 1A suggest that if a systemic AIV strain were to evolve from the LP AIV TX/04 strain, its heat resistance properties in meat from infected chickens probably would be very different from those in artificially infected chicken meat under our assay conditions. Assuming no change in the intrinsic heat resistance of the virus with evolution, inactivation of this hypothetical AIV strain in the meat of infected chickens probably would be similar to that observed for the HP AIV strains PA/83 and Korea/03 because inactivation of TX/04, PA/83, and Korea/03 in liquid medium was similar (Fig. 1B). Therefore, survival curves for AIV TX/04 in artificially infected chicken meat were not constructed.

z-values and regression line equations. The z-value (the temperature increase needed to reduce the D-value by 1 log unit) describes the temperature dependence of a thermal inactivation reaction. A regression plot of log D-value versus temperature yields an equation that can be used to calculate the z-value and predict D-values for additional temperatures. Figure 3 shows regression plots for AIV and NDV in chicken meat. Data previously published for AIV Korea/03 (22) was graphed with the data for AIV PA/83 to allow visual comparison of the two strains. The line equations and z-values for the virus strains used in the present study are listed in Table 3.

As shown in Figure 3, the regression plot for AIV PA/83 inactivation in chicken meat was similar to that previously published for AIV Korea/03. The z-value for AIV PA/83 was 0.5°C lower than that reported for AIV Korea/03 (22), indicating that AIV PA/83 is more sensitive to increasing temperature. For NDV CA/02, stepwise linear regression indicated that meat type (breast versus thigh) did not contribute significantly to the regression model (P = 0.56), and a single line equation was generated from the combined D-value data (thigh meat at all temperatures and breast meat at 57, 59, and 61°C). The regression plots for NDV CA/02 and NDV Ulster were significantly different (Fig. 3). NDV Ulster was more thermostable than NDV CA/02 at 57 to 60°C, but at 61°C the D-values for the two NDV strains were very similar. The higher sensitivity of NDV Ulster to increasing temperature is described by its z-value, which was 1.1°C lower than that for NDV CA/02.

Reduction of AIV and NDV titers during thermal processing of chicken meat. Table 4 shows the predicted reduction in virus titer expected in chicken meat cooked according to current FSIS time-temperature guidelines for a 7-log reduction of Salmonella (24). These estimates are based on the upper limits of the 95% prediction intervals for the D-values, calculated from the data shown in Table 3. As reported previously for the H5N1 HP AIV Korea/03 strain (22), the cooking guidelines are predicted to be effective for inactivation of the AIV and NDV strains used in this study. NDV Ulster was more thermostable at lower cooking temperatures (below 61°C) than the other virus strains tested (Fig. 2 and Table 2), but higher cooking temperatures (especially 70 and 73.9°C) are predicted to be
very effective for the inactivation of each of the virus strains used in this study (Table 4).

In a previous study (17), rapid (≤5 s) inactivation of AIV Korea/03 was found for meat from infected chickens that was incubated in a 70°C thermocycler heating block. The prediction that higher cooking temperatures would also rapidly inactivate the AIV and NDV strains used in this study was tested by incubating meat samples in a 70°C (158°F) or 73.9°C (165°F) thermocycler heating block for very short times (≤5 s). Cooking tests also were repeated with AIV Korea/03-infected meat with a titer 1.2 log EID₅₀/g higher than that tested previously. Detectable virus was inactivated in all of the AIV Korea/03, AIV PA/83, and NDV CA/02 samples during the time that it took the thermocycler block to heat from 25 to 70°C (33 s) or 73.9°C (34 s) (Table 5). Only one of the NDV Ulster samples was positive after treatment, and it had a low virus titer (2.8 log EID₅₀/g). The data from this study indicate that cooking

FIGURE 2. Survival curves for AIV PA/83, NDV CA/02, and NDV Ulster in chicken meat. AIV PA/83 and NDV CA/02 meat samples were taken from infected chickens. NDV Ulster samples were artificially infected by adding the virus to breast meat from uninfected chickens. Breast meat; thigh meat. Each data point represents the average titer of at least three meat samples, and the error bars indicate standard deviations. Detection limit was 2.2 log EID₅₀/g (mean embryo infective dose).
TABLE 2. Experimental D-values for AIV and NDV in chicken meat$^a$

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>AIV PA/83 (breast)$^b$</th>
<th>NDV CA/02 (thigh)</th>
<th>NDV CA/02 (breast)$^b$</th>
<th>NDV Ulster (breast)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D (s)</td>
<td>$R^2$</td>
<td>D (s)</td>
<td>$R^2$</td>
</tr>
<tr>
<td>57</td>
<td>267.6</td>
<td>0.89</td>
<td>221.6</td>
<td>0.91</td>
</tr>
<tr>
<td>58</td>
<td>141.5</td>
<td>0.90</td>
<td>140.2</td>
<td>0.86</td>
</tr>
<tr>
<td>59</td>
<td>81.4</td>
<td>0.88</td>
<td>90.7</td>
<td>0.92</td>
</tr>
<tr>
<td>60</td>
<td>63.4</td>
<td>0.87</td>
<td>52.8</td>
<td>0.95</td>
</tr>
<tr>
<td>61</td>
<td>23.6</td>
<td>0.87</td>
<td>38.4</td>
<td>0.84</td>
</tr>
</tbody>
</table>

$^a$ D-value is the time (seconds) required for a 1-log reduction in virus titer.

$^b$ AIV PA/83 meat samples were taken from infected birds, and inactivation experiments were done in only breast meat.

$^c$ NDV CA/02 meat samples were taken from infected birds, but D-values in breast meat at 58 and 60°C were not determined (ND).

$^d$ Meat samples were artificially infected by adding NDV Ulster to breast meat from uninfected chickens, and inactivation experiments were done in only breast meat.

$^e$ Coefficient of determination.

Temperatures effectively reduce the titer of these AIV and NDV strains in chicken meat and that inactivation is very rapid at higher cooking temperatures.

DISCUSSION

The presence of AIV and NDV in the carcasses and organs of infected poultry is of concern because movement of infected poultry or infected poultry products can spread these viruses to new locations. For example, carcasses or raw meat contaminated with virus could serve as transmission vehicles if fed to poultry or discarded in an area accessible to other susceptible hosts. In January 2007, an outbreak of Asian lineage H5N1 HP AIV occurred on a single turkey farm in Suffolk, United Kingdom. After ruling out several possible sources of the introduction, epidemiologists concluded that importation of fresh turkey meat from a flock in Hungary that was not yet showing clinical signs of HP AIV infection was the most likely source (4). An experimental feeding study demonstrated transmission of Asian lineage H5N1 HP AIV to 3- to 4-week-old White Leghorn chickens that consumed meat with an average dose of 7.8 log EID$_{50}$ per bird, but transmission of an H5N2 HP AIV strain did not occur when chickens consumed infected meat with an average dose of 3.6 log EID$_{50}$ per bird (18). Experimentally, domestic cats have been infected with Asian lineage H5N1 HP AIV after feeding on infected chicken carcasses (14). Various carnivores, including domestic cats, tigers, leopards, stone martens, and dogs, have been infected with Asian lineage H5N1 HP AIV during outbreaks, possibly by consuming infected bird carcasses (9, 15, 16, 21, 27). No comparable feeding studies have been published for NDV-infected meat. However, NDV can be transmitted to chickens by ingestion of feces, and contaminated feed has been implicated in some outbreaks (1). An oral infectious dose of 4 log EID$_{50}$ has been estimated for the HP NDV strain Herts 33/56 in 3-week-old White Leghorn chickens (3), but the dose was given in liquid rather than in meat.

The amount of HP AIV or HP NDV present in meat depends on several variables, such as the virus strain, route of infection, time postinfection, and host factors such as chicken breed, age, and immunity. The highest reported titers for HP AIV in experimentally infected SPF chickens inoculated by the intranasal route (which simulates natural exposure) have been for Asian lineage H5N1 HP AIV strains. Peak meat titers for these strains typically range from 7 to 8 log EID$_{50}$/g in 3- to 4-week-old SPF White Leghorn chickens (an egg-type breed) and are 1 to 2 log EID$_{50}$/g lower in SPF White Rock chickens (a meat-type breed) of the same age (17, 22). Chicken breed had a great-
er effect on the amount of AIV PA/83 found in meat; titers obtained for 4-week-old SPF White Leghorns in the current study were approximately 4 log EID\textsubscript{50}/g higher than those reported for SPF White Rocks of the same age in previous studies (17, 18). In the current study, 5-week-old SPF White Leghorns had PA/83 meat titers approximately 2 log EID\textsubscript{50}/g lower than those in 4-week-old SPF White Leghorns, suggesting that chicken age can affect the meat titer of at least some AIV strains. For HP NDV, there is little published information on virus titers in chicken meat. Alexander et al. (3) reported meat titers of up to 4.2 log EID\textsubscript{50}/g for the HP NDV strain Herts 33/56 in 6-week-old SPF White Leghorns, and meat titers more than 2 log EID\textsubscript{50}/g higher were obtained in the current study for the HP NDV strain CA/02 in 4-week-old SPF White Leghorns. The titers listed here were in meat from sick or dead chickens, and such birds would not be processed for food consumption. Meat titers increase over the course of infection with HP AIV and HP NDV and are expected to be lower in chickens not yet displaying clinical signs of illness (3, 6).

In general, LP AIV and LP NDV are not expected to be present in meat from infected chickens. However, virus from respiratory secretions or feces could be a source of carcass surface contamination. In one study, virus was isolated from body cavity rinses of chickens infected with LP AIV, both before and after the carcasses were soaked in water with 30 ppm of chlorine and chilled, but breast and thigh meat samples taken from these same carcasses were negative for virus isolation (18). This finding suggests that the amount of virus present on meat from properly eviscerated carcasses is quite low. As with virus titers in meat, the concentration of virus in respiratory secretions or feces is variable and depends on several factors. The volume of fluid or amount of feces that could accidentally contaminate the meat during slaughter or evisceration is also variable. However, the virus concentration in meat contaminated in this manner should be lower than that in meat from chickens infected with HP AIV or HP NDV. The artificially infected meat samples used in this study had relatively high titers (6.7 log EID\textsubscript{50}/g for AIV TX/04, 5.4 log EID\textsubscript{50}/g for AIV PA/83, and 7.9 log EID\textsubscript{50}/g for NDV Ulster), comparable to those found in meat from chickens infected with systemic virus strains. Cooking effectively inactivated virus in these artificially infected samples (Fig. 1A and Tables 1 and 5). Therefore, cooking is also expected to inactivate virus in meat that becomes contaminated with AIV or NDV during slaughter and evisceration.

Under the conditions of our assay, an HP AIV strain was inactivated much more rapidly in artificially infected chicken meat than in meat with a similar virus titer taken from infected chickens (Fig. 1A). The infection method was the only variable in these experiments, indicating that at least under some assay conditions the results obtained for HP AIV inactivation in artificially infected meat samples versus meat from infected chickens with a similar titer can be very different. In contrast, HP NDV inactivation in chicken meat was accurately modeled by the artificial infection method used in this study (Fig. 1C). The difference between HP AIV and HP NDV inactivation in artificially

**TABLE 3. The z-values and line equations for thermal inactivation of AIV and NDV strains in chicken meat**

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>z (°C)</th>
<th>Line equation</th>
<th>RMSE\textsuperscript{a}</th>
<th>R\textsuperscript{2d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIV PA/83</td>
<td>4.1</td>
<td>( y = [(0.2458)°(C)] + 16.4339 )</td>
<td>0.08000</td>
<td>0.97</td>
</tr>
<tr>
<td>NDV CA/02</td>
<td>4.8</td>
<td>( y = [(0.2100)°(C)] + 14.3333 )</td>
<td>0.03578</td>
<td>0.99</td>
</tr>
<tr>
<td>NDV Ulster</td>
<td>3.7</td>
<td>( y = [(0.2735)°(C)] + 18.3709 )</td>
<td>0.1229</td>
<td>0.94</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The z-value is the temperature increase required to reduce the D-value by 1 log unit.
\textsuperscript{b} AIV PA/83 and NDV CA/02 meat samples were taken from infected birds. NDV Ulster meat samples were artificially infected by adding the virus to breast meat from uninfected chickens.
\textsuperscript{c} Root mean square error (standard error of the y estimate).
\textsuperscript{d} Coefficient of determination.

**TABLE 4. Log reductions of AIV or NDV titer predicted in chicken meat cooked according to minimum current USDA-FSIS time-temperature guidelines for a 7-log reduction of Salmonella**

<table>
<thead>
<tr>
<th>Temp</th>
<th>D (s)\textsuperscript{b}</th>
<th>Minimum FSIS guideline\textsuperscript{c}</th>
<th>Predicted titer reduction (log EID\textsubscript{50}/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td>°F</td>
<td>AIV PA/83</td>
<td>NDV CA/02</td>
</tr>
<tr>
<td>57.8</td>
<td>136</td>
<td>243.6</td>
<td>184.9</td>
</tr>
<tr>
<td>58.9</td>
<td>138</td>
<td>130.7</td>
<td>108.6</td>
</tr>
<tr>
<td>60.0</td>
<td>140</td>
<td>70.1</td>
<td>63.8</td>
</tr>
<tr>
<td>61.1</td>
<td>142</td>
<td>37.6</td>
<td>37.5</td>
</tr>
<tr>
<td>70.0</td>
<td>158</td>
<td>0.24</td>
<td>0.51</td>
</tr>
<tr>
<td>73.9</td>
<td>165</td>
<td>0.027</td>
<td>0.077</td>
</tr>
</tbody>
</table>

\textsuperscript{a} FSIS, Food Safety and Inspection Service.
\textsuperscript{b} Upper limit of the 95% prediction interval for the D-values, calculated from the line equations + 2(RMSE), shown in Table 3. All of the predictions in Table 4 are conservative estimates based on this number.
\textsuperscript{c} From the time-temperature table for chicken meat with 1% fat.
infected chicken meat versus meat from infected chickens could be due to differences in viral replication in the host. HP AIV antigen can be present inside chicken skeletal muscle cells at levels detectable by immunohistochemistry (11, 13, 23). HP NDV antigen has not been detected, but HP NDV may be in the blood contained within the meat. Very rapid AIV inactivation did not occur in liquid medium containing 10 to 100 times the volume of virus stock added to the artificially infected meat samples (Fig. 1B), which suggests that AIV might not be inactivated so rapidly in artificially infected samples with very high virus titers. However, before heat treatment the artificially infected samples used in this study had AIV titers comparable to those in meat from HP AIV–infected chickens and much higher titers than would be expected in chicken meat contaminated by AIV from respiratory secretions or feces.

All but one of the cooking tests at 70 and 73.9°C were negative for virus isolation (Table 5). Only one of the NDV Ulster samples was positive after cooking, and it had a low titer (2.8 log EID_{50}/g). Positive samples were not expected based on the predictions in Table 4. Given that all of the other NDV Ulster cooking tests performed on the same day with virus from the same working stock aliquot were negative (the 70°C, 5-s samples and the 73.9°C, 0-s samples), it does not seem likely that a subpopulation of heat-resistant virus was responsible for this result. The NDV Ulster titer in the untreated samples (7.9 log EID_{50}/g) was much higher than would be expected in chicken meat contaminated by respiratory secretions or feces. Likewise, the meat samples taken from infected chickens and used for the cooking tests (Table 5) had very high titers, especially the Asian lineage H5N1 Korea/03 strain at 8.0 log EID_{50}/g.

Cooking effectively inactivates AIV and NDV in raw, skinless chicken meat. The meat samples used in this study are “worst case” samples, with higher titers than would be expected in meat from chickens with subclinical infections or in meat contaminated by respiratory secretions or feces. The risk of contaminated meat entering the processing plant can be minimized by implementing strict biosecurity measures and surveillance and detection programs on the farm. Because product composition (such as the amounts of protein and fat) can affect pathogen inactivation, the results described in this study may not apply to other meat products.

TABLE 5. Inactivation of AIV and NDV strains in chicken meat at 70 and 73.9°C

<table>
<thead>
<tr>
<th>Heat treatment</th>
<th>AIV Korea/03 (8.0 log EID_{50}/g)</th>
<th>AIV PA/83 (6.7 log EID_{50}/g)</th>
<th>NDV CA/02 (6.8 log EID_{50}/g)</th>
<th>NDV Ulster (7.9 log EID_{50}/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70°C, 0 s</td>
<td>No. of positive samples^d</td>
<td>Avg titer after treatment (log EID_{50}/g)</td>
<td>No. of positive samples^d</td>
<td>Avg titer after treatment (log EID_{50}/g)</td>
</tr>
<tr>
<td>70°C, 5 s</td>
<td>0</td>
<td>≤2.1</td>
<td>0</td>
<td>≤2.1</td>
</tr>
<tr>
<td>73.9°C, 0 s</td>
<td>0</td>
<td>≤2.1</td>
<td>0</td>
<td>≤2.1</td>
</tr>
<tr>
<td>73.9°C, 5 s</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

^d EID_{50}, mean embryo infective dose. AIV Korea/03, AIV PA/83, and NDV CA/02 meat samples were taken from infected birds. Negative samples were assigned the value 2.1 log EID_{50}/g for the purpose of calculating average titers. The ≤ symbol indicates an average from data that includes at least one negative sample. Detection limit of the assay was 2.2 log EID_{50}/g. ND, not determined.
^b For 0-s treatments, samples were removed immediately after the heating block reached the target temperature.
^c NDV Ulster meat samples were artificially infected by adding virus to breast meat from uninfected chickens.
^d Three samples were tested for each treatment.
^e The titer of the positive sample was 2.8 log EID_{50}/g.

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REFERENCES


