Updated Recommendations for Heat Inactivation of High Pathogenicity Avian Influenza Virus in Dried Egg White for Import/Export Purposes

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High pathogenicity avian influenza viruses (HPAIV) cause severe systemic disease with high mortality in chickens. Isolation of HPAIV from the internal contents of chicken eggs has been reported, and this is cause for concern because HPAIV can be spread by movement of poultry products during marketing and trade activity. This study presents thermal inactivation data for the HPAIV strain A/chicken/PA/1370/83 (H5N2) (PA/83) in dried egg white with a moisture content (7.5%) similar to that found in commercially available spray-dried egg white products. The 95% confidence limits for the survival curves at 54.4°C, 60.0°C, 65.5°C, and 71.1°C were 475.4, 192.2, 141.0, and 50.1 minutes, respectively. The line equation $y = (0.05494/C)^{+6.5693}$ (RMSE = 0.0771) was obtained by linear regression of experimental D-values versus temperature. Conservative predictions based on the thermal inactivation data suggest that standard industry pasteurization protocols would be very effective for HPAIV inactivation in dried egg white. For example, these calculations predict that a 7-log reduction would take only 2.6 days at 54.4°C.

INTRODUCTION

High pathogenicity avian influenza virus (HPAIV) strains cause severe disease with high mortality in chickens and related gallinaceous poultry (14). In chickens, the initial replication site for HPAIV is the respiratory or intestinal tract which is followed by systemic spread of the virus. During the 1983-1984 outbreak of high pathogenicity avian influenza in the northeastern U.S., HPAIV was isolated from oral fluids, albumen, yolk, and the shell surface of eggs obtained from affected flocks in Pennsylvania (4). In one experimental study, HPAIV titers as high as 4.9 log EID$_{50}$ (mean embryonating dose) per ml of egg white were found in eggs from experimentally infected hens (13).

The presence of HPAIV in eggs is a cause for concern because the virus can spread to susceptible poultry via the movement of infected poultry products. Due to the high economic cost of controlling HPAIV in poultry, the World Organization for Animal Health (Office International des Epizooties, OIE), the intergovernmental organization that establishes health standards for international trade of animals and animal products, recommends that poultry products from HPAIV-affected countries, zones, or compartments be treated to inactivate HPAIV prior to export (10). The demonstration of heat inactivation of avian influenza viruses in poultry products suggests that thermal processing could be an effective treatment for many avian influenza virus strains (6, 12, 17, 19).

A previous study performed in our laboratory reported D-values for the HPAIV strain A/chicken/PA/1370/83 (H5N2) (PA/83) in various egg products (13). Using the same procedures were done to determine whether U.S. industry standards for egg product pasteurization, which were developed to inactivate contaminating Salmonella (6), are also sufficient for HPAIV inactivation. The calculations predicted that 15 days would be required to completely inactivate high titer of HPAIV in dried egg white at 54.4°C, rather than the 7-10 days specified by the industry standard. However, the moisture content of the freeze-dried egg white prepared for the previous study was not controlled and was probably much lower than that in commercially available spray-dried egg white products. USDA regulations state that the moisture content of spray-dried egg white must be greater than 6.0% for adequate destruction of Salmonella, and commercially available spray-dried egg white products typically have 6.5-8.0% moisture to provide adequate pathogen kill with an additional margin of safety (5).

For this study, HPAIV-contaminated freeze-dried egg white with an average moisture content of 7.5% was prepared and used for thermal inactivation experiments. A mathematical model for HPAIV inactivation in dried egg white was derived from survival curve data and used to predict the log reductions of HPAIV expected in dried egg white pasteurized according to standard industry protocols.

RESULTS

Survival curves and D-values for PA/83 HPAIV in dried egg white. Figure 2 shows survival curves for PA/83 HPAIV in dried egg white at 54.4°C, 60.0°C, 65.5°C, and 71.1°C. The coefficients of determination ($R^2$) and the D-values calculated from each survival curve are shown in Table 1. A linear model provided a fair-to-good fit for the survival curves, with $R^2$ values of 0.90 or higher for all temperatures except for 65.0°C. As shown in the 65.0°C graph (Fig. 2), the 4-hour time point had an unusually large standard deviation. Similar results were obtained for a second set of triplicate samples. This variability probably accounts for the relatively low values for the 65.0°C curve. For each survival curve, the regression line points include at least one sample in which PA/83 HPAIV was not detected: 0 samples for 54.4°C, 2/3 samples for 60°C, 0/3 samples for 65.0°C, and 2/3 samples for 71.1°C. Negative samples were graphed as 1.7 log EID$_{50}$/g, which is just below the detection limit of the assay (1.8 log EID$_{50}$/g). PA/83 HPAIV was not detected in additional samples incubated at 54.4°C for 2 days (3 samples) or 3 days (9 samples). Regression line equation and the z-value. Figure 3 shows a linear regression plot of log D-value versus temperature for PA/83 in dried egg white. The line equation and RMSD for the linear model are shown in the figure legend. A temperature of 18.2°C was calculated from the graph, with a 95% upper confidence limit of 23.0°C.

DISCUSSION

HPAIV has been isolated in eggs collected during natural outbreaks and experimental infection studies. During the 1983-1984 outbreak of H5N2 HPAIV in the northeastern U.S., HPAIV was isolated from the internal contents of 22% of the chicken eggs sampled from flocks collected in Pennsylvania 1-18 days after clinical signs typical of HPAIV appeared, but the quantity of HPAIV in the samples was not determined (4). One report in the literature describes the isolation of 4.6 to 6.2 log EID$_{50}$/g HPAIV from internal contents of Japanese quail eggs (14) collected during a 2003-2004 H5N1 HPAIV outbreak in Thailand (17), demonstrating that HPAIV may be present in eggs from other susceptible avian species.

In studies with experimentally infected chickens, H5N2 HPAIV was not detected in eggs laid 1-2 DPI, but was isolated from 88% of the eggs laid 3-4 DPI (2). Some of these eggs had virus titers greater than 4 log EID$_{50}$/ml (2), with a maximum titer of 4.9 log EID$_{50}$/ml (13). Liquid egg white is 87.1% water and the density of an egg white is 1.035 g/ml (6). Therefore, 1 ml of liquid egg white would be expected to contain about 0.136 g of dried product with 6.5 to 8.0% moisture. If no loss of virus occurred during the drying process, liquid egg white with 4.9 log EID$_{50}$/ml HPAIV would yield dried egg white with 5.8 to EID$_{50}$/g HPAIV. The dried egg white eggs used in this study had a 5.6 to 0.3 log EID$_{50}$/g HPAIV and could therefore be considered “worst case” samples. Based on the constraints shown in Table 1, pasteurization at 54.4°C for 7-10 days would result in a 19 to 27 log reduction of HPAIV titer. A 7-log reduction, which would reduce an HPAIV titer of 6 log EID$_{50}$/g to one EID$_{50}$/g, takes only 2.6 days at 54.4°C.

In contrast, a previous study done in our laboratory suggested that pasteurization at 54-64°C for 1-10 days would not be sufficient for inactivating high titers of HPAIV in dried egg white (13). The same PAH3/83 HPAIV strain was used for both studies, but the dried egg white used in the previous study had a titer of 7.3 log EID$_{50}$/g (12 J. Beck, unpublished data).