Heat Lability of Five Strains of Infectious Bursal Disease Virus

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ABSTRACT An extensive world trade environment has created a need for many nations to protect their food animal industries against the importation of pathogenic microorganisms. We initiated studies to determine how cooking conditions would affect the viability of different infectious bursal disease virus (IBDV) strains. Five different viral strains were tested. The strains included two serotype 1 variant viruses, Del-A and MD; two serotype 1 classic viruses, STC and D78; and a serotype 2 virus, OH. A 100-µL aliquot of each viral strain in Dulbecco’s Modified Eagle Medium with 2% fetal calf serum was heated to 37, 65, 71, 74, 77, 82, or 100 C for 1 min. Following heat treatment, the virus titers were determined in a BGM-70 cell culture. Virus titers declined following each incubation, and there were no appreciable differences among the five viral strains tested. The greatest decline in titer occurred at temperatures above 65 C. After 1 min at 65, 71, and 100 C, reduction of viable viruses were ≥90, ≥99, and >99.9%, respectively, compared to nonheated controls. A viral reduction curve similar to the first heating trial was observed when viruses were heated at 71 or 74 C for 6 min. Drumsticks and boneless chicken patties were seeded with the virus at a mean value of 10⁵.⁵ TCID₅₀/25µL of IBDV and were cooked to internal temperatures of 71 and 74 C, respectively. All samples were quickly cooled after achieving target temperatures. Viable virus was recovered from both products following cooking.

(Key words: cooking, heat treatment, infectious bursal disease virus, titer)

INTRODUCTION Infectious bursal disease virus (IBDV) causes an immuno-suppressive disease in young chickens (Lukert and Saif, 1997). Following infection, convalescent birds are more susceptible to secondary infections from opportunistic microorganisms. Furthermore, vaccination programs are less effective following IBDV infection.

Infectious bursal disease virus is difficult to inactivate in the environment (Parkhurst, 1964, Benton et al., 1967) and is controlled primarily by vaccination (Lukert and Saif, 1997). Although IBDV is endemic throughout most of the world, several different antigenic and pathogenic types exist in specific geographic locations. For example, antigenic variant viruses have only been identified in North America (Jackwood and Sommers, 1999). Similarly, highly pathogenic strains are found in Europe and Asia but have not been identified in North, Central, or South America. The interstate and international commerce of poultry products, which could contain viable IBDV, threatens the poultry industry (Shane et al., 1994). Minimum commercial cooking temperatures for poultry products are typically 71 to 74 C. Inactivation of IBDV in poultry products at these temperatures has not been tested. In a recent study, IBDV was resistant to temperatures up to 80 C for 1 min (Alexander and Chettle, 1998). In that study, a 50% wt/vol bursal homogenate was combined with peptone broth and then centrifuged to obtain the supernatant that was tested in 1-mL aliquots. In an early study, Cho and Edgar (1969) found that IBDV exposed to 60 C for 90 min still retained nearly 100% infectivity in live birds. In a prior study by Benton et al. (1967) the virus was held at 56 C for as long as 5 h. In that study, a titer of 10¹.⁷8 egg infectious dose (EID₅₀)/0.1 mL remained after heat treatment as compared with 10¹.⁷⁸ EID₅₀/0.1 mL for the control, demonstrating no apparent difference. In another study by Landgraf et al. (1967) it was shown that IBDV exposed to 70 C for 30 min was apparently neutralized. They used vials of sterile, milli-pore-filtered kidney and bursa organ suspension that were placed in a hot water bath for the heating trial. In the present study, we used higher temperatures than
previously reported (up to 100°C). Inactivation of IBDV within poultry products has not been investigated. Consequently, this aspect of our study involved exposing viral-inoculated poultry products to two different cooking conditions.

**MATERIALS AND METHODS**

**Viruses and Cells**

The IBDV strains tested included D78, STC, Del-A, MD, and OH. The D78 (Jackwood et al., 1985) and STC (Rosenberger et al., 1975) are classic strains of serotype 1 IBDV (Lukert and Saif, 1997). Del-A (Rosenberger et al., 1985) and MD (Saif, 1984) are variant strains of serotype 1 IBDV. OH (Jackwood et al., 1982) is a nonpathogenic (Ismail et al., 1988) strain of serotype 2 IBDV (Barnes et al., 1982).

The cell line used to culture IBDV was BGM-70 (Jackwood et al., 1987) which originated from baby Grivit monkey kidney cells (Lukert et al., 1975). Cells were propagated in Dulbecco’s Modified Eagle Medium (DMEM) with 10% fetal calf serum according to the procedures of Jackwood et al. (1987). BGM-70 cell cultures with 2% fetal calf serum were infected and frozen-thawed three times before storing at −70°C in 1-mL aliquots.

**In-Vitro Heating Block Study**

To study the heat resistance of IBDV, 100 µL of each of the five strains of IBDV in DMEM with 2% fetal calf serum were placed in individual 0.5-mL microcentrifuge tubes. The microcentrifuge tubes were placed in a Perkin Elmer 2400 Gene Amp PCR system that was programmed to ed temperatures and hold for 1 min then drop rapidly to 4°C. The temperatures set were 37, 65, 71, 74, 77, 82, and 100°C. A separate run was performed for each of the five strains at 71 and 76°C for 1, 2, 3, 4, 5, and 6 min. The heat-exposed virus was stored at −70°C until it was tested for viability in 96-well culture plates containing BGM-70 cells. Concurrent negative control samples were obtained from uninfected BGM-70 cell cultures containing DMEM with 2% fetal calf serum. The titer of viable virus was determined and reported as the quantity that produced cytopathic effects in 50% of the wells containing BGM-70 cells (TCID₅₀/25mL).

**Viral Recovery**

All cooked, uncooked, inoculated, and uninoculated chicken drumsticks were ground with a stainless steel model 4822 Hobart meat chopper that was sterilized in an autoclave between each sample. All the boneless chicken patties were homogenized in 22 mL of DMEM solution containing 2% fetal calf serum in sterile whirlpak bags by using a Stomacher Lab-Blender 400. The homogenate was centrifuged until an aqueous fraction appeared at the surface. This aqueous fraction was collected and saved. A similar procedure was used to prepare homogenates from the ground chicken drumsticks.

Homogenates were extracted with an equal volume of chloroform prior to examination for viable virus. Fungizone (250 µL/mL) and gentomicin reagent solutions (100 µg/mL) were added to each sample, and a 3-mL volume was used to inoculate 25 cm² tissue culture flasks containing BGM-70 cells. The presence of live virus in the samples was identified by cytopathic effects visualized by microscopic examination of the tissue cultures.

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and confirmed by the absence of cytopathic effects in controls from uninoculated product samples.

**RESULTS**

All five IBDV strains, suspended in DMEM cell culture medium, held for 1 min at temperatures below 71 C, showed relatively small reductions in titer as compared to temperatures above 71 C (Figure 1). Virus held at 37 C for 1 min had $10^{0.25}$ log$_{10}$/25 L TCID$_{50}$ or less reduction in titer compared to the unheated controls held at 4 C (Figure 1). Between $10^{1.0}$ and $10^{2.5}$ log$_{10}$/25 L TCID$_{50}$ of the initial $10^{4.0}$ to $10^{6.0}$ log$_{10}$/25 L TCID$_{50}$ viral titer remained of all five strains after this 37 C incubation. After a 1-min incubation at 65 C, the reduction of viral titers ranged between $10^{1.0}$ and $10^{2.5}$ log$_{10}$/25 L TCID$_{50}$ of virus remaining after exposure to 82 C for 1 min but was reduced to $10^{0.25}$ log$_{10}$/25 L TCID$_{50}$ after exposure to 100 C. Between $10^{0.25}$ and $10^{0.75}$ log$_{10}$/25 L TCID$_{50}$ of all strains tested remained viable after exposure to 100 C for 1 min.

The stability of IBDV strains was examined at two temperatures (71 and 74 C) typically used as minimum processing endpoint temperatures by commercial poultry processors. Viability following exposure to these temperatures was examined for up to 6 min. A 30 to 50% titer reduction was observed within the first min (Figure 2). Less than $10^{0.75}$ log$_{10}$/25 L TCID$_{50}$ of additional reduction in titer was observed following exposure to 71 or 74 C for an additional 2 to 6 min. Depending on the virus sample, between $10^{0.75}$ to $10^{2.5}$ log$_{10}$/25 L TCID$_{50}$ of virus still remained after 6 min at 74 C (Figure 2).

Cytopathic effects typical of IBDV infection were observed in BGM-70 cell cultures for all five strains of IBDV recovered from inoculated drumsticks that were cooked in hot oil. Similar results were observed for boneless chicken patties cooked in a steam-injected flame grill (Table 1). No cytopathic effects were observed in cell cultures with uninoculated control drumstick homogenates or uninoculated control chicken patty homogenates.

**DISCUSSION**

The infectious bursal disease virus suspended in DMEM cell culture medium with 2% fetal calf serum retains a residual titer sufficient to infect cell cultures after exposures to 37, 65, 71, 74, 77, 82, and 100 C for 1 min. Viral titers that remained after heating to 100 C were between $10^{0.25}$ to $10^{0.75}$ log$_{10}$/25 L TCID$_{50}$ (Figure 1). The steepest decline in titers was observed between 65 and 77 C (Figure 1). Above 77 C, the titer reduction became more gradual. The STC strain appeared to be more affected than the other strains by temperatures above 77 C with a titer of $10^{3.25}$ log$_{10}$/25 L TCID$_{50}$, which fell to $10^{0.25}$/25 L TCID$_{50}$ after exposure to 100 C for 1 min. The results of our study compare well with a study by Alexander and Chettle (1998), in which they reported sharp titer drops during the first 2 min at temperatures between 70 and 80 C and a relatively flat curve beyond 2 min. A study by Landgraf et al. (1967) demonstrated no IBDV growth in embryonated chicken eggs after a 30-min exposure of the virus to 70 C.

The USDA-Food Safety Inspection Service’s minimum internal temperature requirement is 71 C for poultry products that do not have curing agents added (USDA food safety hotline). Internal product temperatures in this study met and exceeded the USDA requirement. Minimum internal temperatures of 71 and 74 C are commonly used for preparation of commercial poultry products. Chicken drumsticks and boneless chicken patties were seeded with virus in this study and had enough viable IBDV remaining after cooking to infect and cause cytopathic effects in BGM-70 cells. The quantity of IBDV in chicken muscle during an active infection is not known. It is likely that it is a very small amount. Thus, the titers used in this study may be artificially high and do not reflect actual conditions. However, the amount of virus in the processed chicken patties could be much higher due to contamination with IBDV-infected bursa tissue that remains in the carcass. In this study we also tested both temperatures in vitro for up to 6 min and found that after 6 min at 71 or 74 C, all five IBDV strains tested had titers between $10^{0.75}$ and $10^{2.25}$ log$_{10}$/25 L TCID$_{50}$. Much of the titer reduction occurred during the first 2 min. After 2 min, only a small additional reduction of titer occurred with Del-A, MD, and OH serotypes, and no further reduction was observed after 2 min with STC. The D78 serotype had no additional reduction in titer after 3 min (Figure 2).

The relative heat resistance of IBDV and its ability to survive 60 or more d in poultry litter (Vindevogel et al., 1976) creates some important epidemiological considerations. Possible implications of this have been shown by development of IBDV antibodies found in Emperor and...
Adelie penguins in Antarctica (Gardner et al., 1997). It has been suggested that poultry product scraps, footwear, clothing, or equipment from researchers and visitors to Antarctica may have provided an entry opportunity of the virus to the continent. Also, countries, such as New Zealand, that have been trying to remain IBDV free, impose strict poultry product importation guidelines. This restriction could become a more global trend by other nations protecting their poultry industries from antigenic variants and highly virulent viruses.

Product palatability is an important consumer requirement. The time required to completely inactivate IBDV at 71 and 74 C cooking temperatures appears to be greater than 6 min. Higher temperatures or longer cooking times needed to totally inactivate IBDV may compromise the texture and palatability of poultry products, which could likely make total heat neutralization of IBDV impractical for commercial poultry products. An adequate viral reduction in poultry products would be ideal that would preserve consumer appeal and reduce the risk of spreading virus to an acceptable level. This will be a point of debate to be resolved by new risk analysis regulations currently being drafted by countries involved in international food commerce.

### TABLE 1. Cytopathic effect observations from virus spiked poultry products

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Drumsticks</th>
<th>Boneless patties</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control¹</td>
<td>Spiked²</td>
</tr>
<tr>
<td>D78</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Del-A</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>MD</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>OH</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>STC</td>
<td>–</td>
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</tr>
</tbody>
</table>

¹− = No cytopathic effect; + = cytopathic effect observed as rounded and detached cells that had a ground glass appearance.

²Control products underwent cooking and culture procedures identical to spiked products.

³Spiked products had 300 µL viral culture introduced into the deepest part of the tissue with a 22-ga, 1.5-inch hypodermic needle.
The prevalence of IBDV in slaughter age chickens is not known, thus the potential for IBDV to contaminate poultry products needs to be studied. Poultry offal processing may not adequately neutralize IBDV before it is added as a component of animal feed. The possibility that IBDV could be spread by feed containing poultry by-products should be considered. Acceptable uses of irradiation and other methods to further inactivate IBDV in animal feeds and poultry products may need to be considered.

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REFERENCES