Reduction of Thermotolerant **Campylobacter** Species on Broiler Carcasses following Physical Decontamination at Slaughter

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**ABSTRACT**

To reduce the incidences of human *Campylobacter* infections, a number of countries are investigating methods for reducing human exposure to *Campylobacter* from broiler meat. In addition to implementing biosecurity measures at the farm, *Campylobacter* may be controlled by reducing *Campylobacter* counts through physical decontamination of the meat. The current study was conducted to compare the *Campylobacter*-reducing ability of three physical decontamination techniques, forced air chilling, crust freezing, and steam-ultrasound, performed in the plant with naturally contaminated broiler chickens. The effects of all three techniques were evaluated and compared with the effect of freezing. Mean reductions obtained were 0.44 log CFU per carcass, 0.42 log CFU per sample, and ≥2.51 log CPU per carcass, respectively. All techniques resulted in significant reductions of the *Campylobacter* concentration on the carcasses ($P < 0.05$). However, none of the techniques were as effective as freezing based on reductions in *Campylobacter* counts and on adverse effects. The increase in *Campylobacter* counts on carcasses following visceral rupture during the evisceration operation also was examined. Visceral rupture resulted in an increase of 0.9 log CFU per carcass, suggesting that *Campylobacter* counts also may be reduced by optimizing the hygienic design of equipment or by physical removal of fecal contamination.

*Campylobacter* spp. continue to be the most common cause of human gastrointestinal disease in Europe, as in preceding years (19). In risk factor studies, poultry meat (20, 34), especially fresh and unfrozen broiler meat, has been identified as the major risk factor for campylobacteriosis (48). As a consequence, several countries have developed quantitative risk assessments to support management decisions regarding the control of this pathogen (24, 29, 32, 33, 39). These risk assessments suggest that reduction of the *Campylobacter* concentration on broiler chicken meat will impact considerably the number of human cases of campylobacteriosis.

Although good hygienic practices during rearing and processing of broilers may reduce the occurrence of *Campylobacter*, total elimination of the organisms from live poultry and poultry meat is not possible through hygienic measures alone (2, 12, 22). To obtain a further reduction, various decontamination techniques must be used.

Chemical decontamination of broiler carcasses has been used in the United States for several years. Some of the most commonly used antimicrobial substances are acidified sodium chloride, chlorine, chlorine dioxide, trisodium phosphate, cetylpyridinium chloride, ozone, and peroxyacetic acid (36, 37). In the European Union, chemical decontamination of foods of animal origin has been allowed since Regulation (EC) No. 853/2004 (13) came into force in January 2006. However, no such chemicals have actually been authorized by the Commission because none of the substance applications have been approved by the European Food Safety Authority because of insufficient documentation (14–18).

Several physical decontamination techniques, including freezing, irradiation, and steam, have been effective against *Campylobacter*. Using these techniques, as little as a 0.5-log reduction in *Campylobacter* counts and as great as total elimination of *Campylobacter* has resulted (9, 21, 30, 41, 43, 49). Each method has its advantages and disadvantages with relation to appearance of the final product, consumer acceptance, price, etc. For example, although irradiation is very effective, it is expensive and will meet considerable resistance from consumers, especially in Europe (10, 23, 47). Therefore, this decontamination technique is not considered a viable management option. Freezing of broiler meat is also effective for obtaining marked reductions in *Campylobacter* counts, and researchers have predicted that application of this technique will significantly decrease the incidence of human campylobacteriosis (39). Freezing already has been implemented as an intervention in Iceland, Norway, and Denmark (21, 25). In Denmark, this intervention has been used on a voluntary basis where practical and possible. However, freezing of meat from all *Campylobacter*-positive broiler flocks in Denmark is not a feasible option because it would limit the marketing of domestically produced chilled broiler meat during periods when *Campylobacter* prevalence is high. Consequently, the import of chilled meat would have to increase to satisfy consumer demands. Because imported meat historically has had a higher *Campylobacter* prevalence than found in domestic product (4), the *Campylobacter* contamination problem in chilled chicken meat sold in Denmark would be aggravated further. Significant *Campylobacter* reduction and mainte-
nance of product quality has been difficult to achieve with steam treatment because of the boiled appearance of the skin or meat surface (28, 46).

Ideally, an appropriate physical decontamination technique would be acceptable to consumers while still leaving the meat fresh with no change in product quality. To date, only forced air chilling and crust freezing meet these criteria. However, the effectiveness of these methods against Campylobacter has been variable between studies. A new decontamination technique, Sonosteam, is being developed based on simultaneous treatment of the meat surface with steam and ultrasound. Ultrasound enhances the killing effect of steam by efficiently removing the protective air on the meat surface. However, the effectiveness of this method against Campylobacter has not been investigated.

Because fecal contamination of carcasses during slaughter increases the concentration of Campylobacter (8) on these carcasses, reducing the incidence of fecal contamination would be a useful supplement to physical decontamination methods.

The aim of this study was to investigate the reduction of concentrations of naturally occurring thermotolerant Campylobacter species on broiler carcasses during industrial processing after the in-plant application of three decontamination methods: forced air chilling, crust freezing, and steam-ultrasound. The results obtained were to be used in a quantitative risk assessment of the cost-effectiveness of selected interventions during broiler production. The reductions obtained by forced air chilling, crust freezing, and steam-ultrasound were compared with reductions obtained by freezing (data from a previous study). The increase in Campylobacter counts on carcasses after visceral rupture during evisceration also was examined.

MATERIALS AND METHODS

Broiler flocks. The study included carcasses and breast fillets from Campylobacter-positive broiler flocks processed on different days at a Danish slaughter plant. One week before slaughter, the flocks were determined to be Campylobacter positive by PCR analysis of sock samples (31). Each decontamination technique was tested in duplicate or triplicate, i.e., on two or three flocks processed on different days.

Forced air chilling. Carcasses were chilled in a forced air chiller in accordance with plant procedures. The carcasses were carried through the forced air chiller on a continuous shackle line for 3 h to obtain an outer carcass temperature of approximately 3°C. In total, 50 carcasses were collected for analysis from each of three flocks both before (controls, n = 25) and after (n = 25) treatment.

Crust freezing. Skinless breast fillets treated in a continuous CO₂ belt freezer were fed evenly into the low temperature–freezing zone (−55°C) via the loading freezer belt. Fillets were crust frozen individually and reached an outer surface temperature of approximately −1°C after treatment, just before packaging. In total, 100 breast fillets from each of three flocks were collected for analysis: two fillets per sample, 25 control samples (before treatment), and 25 treated samples.

Steam combined with ultrasound. Carcasses were treated with steam in combination with ultrasound using a recently developed Sonosteam technique (patent DK/28.03.2001/ DKA200100514). The technique was applied after evisceration but before the inside-outside bird washing procedure. A proof-of-concept treatment apparatus not developed for in-line treatment was used. The equipment was located in a container outside the plant. Therefore, carcasses were sampled inside the plant, placed in sterile plastic bags, and carried outside to the container for immediate treatment. Whole carcasses were treated individually in a treatment chamber mounted with a row of specially designed nozzles that supplied steam simultaneously with the generation of ultrasound waves for outside treatment, and a rod with nozzles was used for inside treatment. Each carcass was hung on rotating shackles and treated inside for 5 s and outside for 10 s. In total, 60 carcasses from each of two flocks were collected for analysis before (controls, n = 30) and after (n = 30) treatment.

Visceral rupture during evisceration. To examine the increase in Campylobacter contamination of carcasses due to visceral rupture during evisceration, carcasses with (n = 25) and without (controls, n = 25) visual fecal contamination were sampled after evisceration. In total, 50 whole carcasses were collected for analysis from each of three flocks.

Sample preparation. Carcasses were prepared as described by the U.S. Food and Drug Administration (44) with minor modifications. Each carcass was placed in a 3,500-ml stomach bag with a filter (Bie & Berntsen A/S, Rødovre, Denmark), and 200 ml volume of 0.1% buffered peptone water (BPW) was added, each liter of which contained 1.0 g of peptone (BD 211677, Merck, Darmstadt, Germany), 17.5 g of sodium chloride (1.06404.1000, Merck), 3.5 g of disodium hydrogen sulfate (1.06404.1000, Merck), and 1,000 ml of distilled water. The bag was then sealed and the contents were manually massaged for 2 min. The bag was then tilted to let the liquid flow to one bottom corner, which was sanitized with 70% ethanol and then cut off with sterile scissors. Holding back the carcass and the filter, the rinsate was poured into a 250-ml sterile centrifuge tube, which was kept at 4°C for a maximum of 24 h before analysis. The chilled rinsate was then centrifuged at 13,000 × g for 15 min, the supernatant was discarded, and the pellet was resuspended in 10 ml of 0.1% BPW.

Breast fillets were similarly prepared by surface rinsing. Two fillets were placed in a 400-ml stomach bag with a filter (Bie & Berntsen A/S), and 50 ml of 0.1% BPW was added. The sample was treated as above, except with a 50-ml sterile centrifuge tube and 5 ml of 0.1% BPW.

Microbiological analyses. Naturally occurring thermotolerant Campylobacter species in the chicken rinse were enumerated in accordance with the direct plating technique described by Rosenquist et al. (40). Ten-fold dilutions of the chicken rinsates were made in BPW, and 0.1 ml of the dilutions was plated onto Abeyta-Hunt-Bark agar (44) containing 0.1% triphenyl tetrazolium chloride for red staining of colonies.

Statistical analysis. Before analysis, bacterial counts (CFU per sample) were log transformed to approximate a normal distribution of the data. Samples in which Campylobacter was present but below the detection limit were given a value of one-half of the detection limit. An analysis of variance was carried out using PROC GLM within the SAS Enterprise Guide statistical software, version 3.0 (SAS Institute Inc., Cary, NC). An α value of 0.05 was used as the level of significance.
TABLE 1. Campylobacter concentrations in samples from broiler flocks before (control) and after (treated) treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flock 1</th>
<th>Flock 2</th>
<th>Flock 3</th>
<th>Flock 4</th>
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<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
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<tr>
<td>Mean</td>
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<td>25</td>
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<td>25</td>
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<tr>
<td>Crust freezing</td>
<td>3.36 A</td>
<td>25</td>
<td>2.91 C</td>
<td>25</td>
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<td></td>
<td>25</td>
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<td>24</td>
<td></td>
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<tr>
<td>Steam-ultrasound</td>
<td>3.60 A</td>
<td>30</td>
<td>1.67 b</td>
<td>29</td>
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<td></td>
<td>30</td>
<td></td>
<td>29</td>
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<tr>
<td>Freezing d</td>
<td>2.59 A</td>
<td>30</td>
<td>1.01 b</td>
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<tr>
<td>Mean log reduction</td>
<td>0.44</td>
<td>0.20</td>
<td>0.42</td>
<td>0.03</td>
</tr>
</tbody>
</table>

a Within a row, means with different letters are significantly different (P = 0.05).

b Two samples treated with steam ultrasound and 14 treated by freezing had values below the detection limit. Those samples were given values of one-half of the detection limit and were included in the analysis.

c ND, not done.
d Data from Rosenquist et al. (40).

RESULTS

Effects of decontamination techniques. All decontamination techniques investigated resulted in significant mean reductions of the Campylobacter concentrations on broiler carcasses (P < 0.05). The mean concentrations before and after treatment and the mean reductions for each studied flock are given in Table 1. All control samples were positive for Campylobacter, and all flocks were positive for Campylobacter 1 week before slaughter. Therefore, all samples were assumed Campylobacter positive before treatment. Because Campylobacter colonization of all broilers in a flock can occur within a few days (3, 38, 42, 45), this assumption was considered reasonable.

Forced air chilling resulted in a significant mean reduction of 0.44 log CFU per sample. However, the reductions obtained within each flock differed significantly from 0.03 to 0.69 log CFU per carcass. Along with a significant flock × treatment interaction, these differences indicated that the effect of forced air chilling was not consistent between flocks. Comparisons of treatment groups indicated a significant difference only between flocks 2 and 3 (Table 1).

Crust freezing caused a significant mean reduction of 0.42 log CFU per sample. The standard error of the mean (SEM) for the three investigated flocks was low (Table 1), indicating that the reduction obtained by this process was consistent. This conclusion was supported by the lack of a significant interaction between the flock and treatment variables.

The steam-ultrasound treatment resulted in reductions of ≥2.51 log CFU per carcass. The exact reductions could not be determined because counts from 2 of the 30 samples from flock 1 were below the detection limit after treatment. The reductions obtained for the two flocks significantly differed (Table 1), as was the flock × treatment interaction (P < 0.05). An adverse effect of this technique was a slightly boiled appearance of the carcass skin.

For each of the decontamination techniques investigated except forced air chilling, the initial mean Campylobacter concentration on the carcasses or fillets was significantly different between flocks.

Effect of visceral rupture. A higher mean concentration of Campylobacter (0.9 log CFU per carcass) was visually found on carcasses contaminated with fecal material than on those without such material (P = 0.05). Interactions between flock and treatment were not significant. The SEMs for the three flocks tested were low (Fig. 1), indicating that the increase in concentration caused by fecal contamination during evisceration was fairly uniform between flocks.

Summary. Mean reductions of 0.44, 0.42, and ≥2.51 log units were obtained by forced air chilling, crust freezing, and steam-ultrasound treatments, respectively. The steam-ultrasound method was the most effective decontamination method. Visceral rupture yielded an increase in Campylobacter of 0.9 log units. Therefore, as an alternative or supplement to physical decontamination, measures to reduce fecal contamination with Campylobacter should be considered.
DISCUSSION

All of the investigated decontamination techniques resulted in reductions of the concentration of naturally occurring Campylobacter species on the carcasses and fillets, with some variation in the reduction achieved. In a risk assessment, Rosenquist et al. (39) estimated that a 2-log reduction would result in a significant reduction in the number of human campylobacteriosis cases associated with broiler meat. Therefore, a reduction of this magnitude would be desirable for increasing the safety of Campylobacter-contaminated broiler chickens. A 2-log reduction in Campylobacter counts has been obtained by freezing the carcasses (9, 21, 30, 43, 49). In an earlier Danish study, the immediate mean reduction after in-plant freezing was 1.44 log units, although according to published reports the Campylobacter counts will decrease further with time. The decrease during storage will occur at a slower rate than that during the freezing procedure (9, 21, 41, 43).

Of the investigated techniques, steam combined with ultrasound applied before washing achieved the greatest reductions of Campylobacter, even greater than those obtained by freezing. However, a large amount of variation between replicates (flocks) was observed. Possible explanations for this could include instability of the equipment and the fact that we were not able to register the exact reduction of the first examined flock with the lowest initial Campylobacter concentration because the values for several of the samples after treatment were below the detection limit. The carcasses appeared to be slightly boiled after treatment. Adjustments to the equipment and treatment time were needed during the development process. Observed initial reductions of Campylobacter obtained by the steam-ultrasound treatment were similar to results reported previously for steam treatment (100°C) for 10, 12, and 20 s at atmospheric pressure (28). In that study, the carcasses also appeared to be boiled after steam treatment (28). Therefore, steam treatment is not a viable alternative to the steam-ultrasound technique. According to another study, steam treatments at 90°C (atmospheric pressure) for up to 24 s (46) caused smaller reductions of Campylobacter than did treatment at 100°C.

Ultrasound treatment creates an enhanced penetrating effect of steam by removing any protective surface boundary layer of air or vapor present around an object, thus allowing the steam to reach the bacteria in the microstructure and cavities on the meat surface more efficiently. This combination optimizes the steam treatment, allowing for a shorter treatment time at a high temperature. This combination has the advantage of the killing effect of steam without affecting the outer layers of the epidermis. Preliminary results with improved equipment developed for in-line industrial use have indicated minor but acceptable changes in the appearance of the poultry skin. The Campylobacter reductions obtained, however, have not been of the same magnitude as those in the present study, probably because the treatment time was reduced to 1 to 2 s. Although the steam-ultrasound technique has provided promising results, more studies are needed using in-line equipment to determine the actual effect on an industrial scale.

Crust freezing is a relatively new technique for rapid chilling of meat, and few microbiological studies of this technique have been published. The investigated CO2 crust freezing technique produced consistent approximately 0.5-log reductions in Campylobacter concentrations. Corry et al. (11) found that crust freezing of chicken carcasses could be very effective for reducing Campylobacter counts (≥2 log units), although the exact technique investigated was not specified. Various methods can be used for crust freezing, e.g., cryogenic freezing with cryogens N2 or CO2 or impingement freezing with cold high-velocity air. Each method could provide different results. In general, the technique is based on rapid ice crystallization on the meat surface, resulting in a thin frozen crust, followed by temperature equalization. Because Campylobacter is primarily a surface contaminant and is reduced by 0.5 to 2 log units during the temperature drop of ordinary freezing (9, 21, 41, 43), crust freezing could have additional decontamination potential. The reason for less dramatic reductions compared with ordinary freezing might be the fact that rapid freezing causes smaller ice crystals and thus less damage to the bacterial cells than occurs with ordinary freezing.

Ambiguous results have been obtained when comparing forced dry air chilling with immersion chilling for reducing Campylobacter counts (1, 7, 26, 40). However, various studies have indicated that chilling has a Campylobacter-reducing effect. In the present study, Campylobacter reductions of 0.03 to 0.69 log CFU per carcass were achieved after forced air chilling. This reduction was less than that documented in other studies: 0.8, 1.4, and 0.83 log CFU (1, 26, 40). The high level of variation in our data implies that the effect of this technique was not consistent between replicates (flocks). The flocks were examined on different days, and the variation might be explained by adjustments to or variations in production parameters, i.e., time, temperature, and skin desiccation. Because the initial Campylobacter concentrations were not significantly different between flocks in the chilling experiments, initial concentration was not considered an influencing factor. If this process in general is to be relied on as an intervention step, more investigation and documentation demonstrating reliability and repeatability will be required.

The reductions obtained with crust freezing and forced air chilling were unable to meet the 2-log reduction recommendation of the Danish risk assessment (39). One approach to reducing the Campylobacter counts further would be to combine the techniques (multiple hurdle approach) to achieve synergistic or additive effects. Published studies on this subject in relation to Campylobacter are sparse. James et al. (28) combined steam or hot water with rapid cooling, chilling, or freezing and documented greater reductions when using combined treatments than when using individual treatments. However, the data were not presented in such a way as to allow evaluation of whether the effects were synergistic or additive.

In the present study, carcasses visually contaminated with fecal material after the evisceration process had sig-
nificantly higher *Campylobacter* counts than did carcasses without visual contamination. These findings are in accordance with those of Berrang et al. (8), who found increasing *Campylobacter* contamination on carcasses with increasing amounts of fecal material. Hygienic measures could likely reduce this contamination, for example by optimizing the hygienic design of equipment to physically remove fecal contamination (e.g., by extra washing of carcasses). The frequency of intestinal rupture differs between plants and flocks. The target in most Danish plants is a rupture frequency of less than 5%, although the actual frequency may occasionally be higher. However, an increased effort during processing will not reduce the overall level of *Campylobacter*. The reduction would apply only to the evisceration segment of the production line. Nevertheless, a reduction in this type of *Campylobacter* contamination would likely have great impact on the risk of illness, because highly contaminated meat constitutes a higher risk (24, 29, 32, 33, 39). Berrang et al. (5) suggested that it may be more efficient to intervene in the feather-removal process, where the carcasses also are contaminated with fecal material. Studies of the amount of contamination that occurs during the defeathering step all revealed increased contamination (6, 27, 35). However, a practical way to markedly reduce fecal contamination during processing has not yet been developed.

This study has generated information on the effectiveness of different physical decontamination techniques for reducing counts of thermotolerant *Campylobacter* strains on naturally contaminated chicken carcasses during processing. The techniques resulted in reductions of 0.4 to 2.5 log units and could be effective decontamination techniques for *Campylobacter* reduction when used alone or in combination. The steam-ultrasound method was the most effective of the three decontamination methods investigated. However, no method was as efficient as freezing when evaluating reductions in *Campylobacter* counts without adverse effects. This study also revealed the need for improved hygienic practices. However, no definitive methods were proposed.

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