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The influence of freezing and duration of storage on *Campylobacter* and indicator bacteria in broiler carcasses

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Abstract

In total, 215 commercially processed broiler carcasses were examined to determine optimum cultural enumeration, the effects of freezing, method of thawing, and duration of frozen storage on levels of *Campylobacter* spp. and fecal coliforms. Enumeration studies compared MPN procedures to direct plating onto selective mCCDA agar and indicated equivalency for quantitation of *Campylobacter* spp. Levels of *Campylobacter* and fecal coliforms were subsequently estimated by direct plating of carcass rinses. Freezing of naturally contaminated carcasses followed by storage at −20 °C for 31, 73, 122 and 220 days showed statistically significant ($P \leqslant 0.05$) reductions in *Campylobacter* counts initially as compared with counts found on fresh product. Among 5 lots of broilers, levels of *Campylobacter* on carcasses were reduced by log mean values ranging from 0.65 to 2.87 after freezing and 31 days of storage. Similar reductions due to freezing were not observed for fecal coliforms counts. The level of *Campylobacter* was reduced by approximately one log immediately after freezing, and remained relatively constant during the 31–220 days of frozen storage. The levels were constant during 7 days of refrigerated storage. After 31 days of frozen storage there was a reduced rate in reduction of counts among broilers thawed at 7 °C as compared to thawing at 22 °C with either cultural method (MPN and mCCDA). These findings warrant consideration of the public health benefits related to freezing contaminated poultry prior to commercial distribution to reduce *Campylobacter* exposure levels associated with contaminated carcasses.

Keywords: *Campylobacter*; Fecal coliforms; Enumeration; Freezing/thawing/chilling; Storage; Poultry

1. Introduction

Campylobacter jejuni is the most frequent cause of foodborne bacterial infection in many developed countries (Altekruse et al., 1999; Friedman et al., 2000). The organism has been isolated from various food sources such as poultry, red meat, and raw milk (Atanassova and Ring, 1999). Epidemiological studies indicate that handling raw poultry or eating undercooked poultry are important risk factors for transmitting campylobacteriosis in many industrialized countries. Additional identified risk factors for humans include consumption of meats other than poultry, drinking untreated surface water, or drinking unpasteurized milk and dairy products (Friedman et al., 2000).

High frequencies and levels of *Campylobacter* spp. in poultry products have been reported among numerous developed countries. Atanassova and Ring reported an incidence of 45.9% contamination in broiler carcasses in Germany (1999). Ono and Yamamoto reported a prevalence of 45.8% in domestic retail poultry in Japan (1999). Madden and co-workers reported a 38% frequency in cut-up chicken portions in Northern Ireland (1998). The Icelandic surveillance program for Campylobacter

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reported an annual flock prevalence of 13.7–19.2% in ceaca samples from processed broiler flocks from 2000 to 2004 (Icelandic Veterinary Services, 2004). Various levels of *Campylobacter* have been reported in poultry products. Berrang et al. (2001) reported concentrations ranging from 2 to 3 log 10 cfu g^{-1} of *Campylobacter* in breast skin, thigh skin and drumstick skin samples and significantly lower concentration in the corresponding meat samples. Whyte et al. (2001) recovered 1.88–3.59 log 10 cfu g^{-1} of *Campylobacter* from neck skins, depending on the makeup of a water-bath in which poultry carcasses were immersed. Stern and Robach (2003) reported the average level of *Campylobacter* as $10^{4.11}$ cfu $carcass^{-1}$ in 1995 being reduced to$10^{3.05}$ cfu $carcass^{-1}$ in 2001 among north Georgia (United States), processed whole broiler rinses. Although the levels were greatly reduced, the frequency remained quite high, with approximately 85% of the carcasses sampled being contaminated.

A positive relationship between the exposure to pathogenic *Campylobacter* cells and the probability of infection has been published (Black et al., 1998). This relationship suggests that a significant reduction of viable *Campylobacter* spp. in commercial poultry products could result in a reduced incidence of *Campylobacter* infections in humans. A number of chemical or physical decontamination methods have been investigated to control the level of *Campylobacter* and other human pathogens on broiler carcasses including ozonation, super-chlorination, organic acids and steam pasteurization (Whyte et al., 2003).

While *Campylobacter* spp. routinely demonstrate survival on broiler carcasses held under refrigeration (Stern, 1995), freezing is known to decrease the viability of some bacteria including *Campylobacter*. The loss of viability has been attributed to ice nucleation and dehydration. Stead and Park (2000) demonstrated that *Campylobacter* encounter oxidative stress during freezing and thawing and that superoxide dismutase plays an important role as a resistance mechanism for cells to overcome this stress. Studies, both in pure broth cultures (Humphrey and Cruickshank, 1985) and on naturally contaminated broiler carcasses (Stern et al., 1985), ground beef livers (Hänninen, 1981) and artificially contaminated beef trimmings (Moorhead, 2002), which were frozen and thawed, showed reductions in levels of *C. jejuni* or *C. coli*. Moore et al. (2002) observed that 94% of fresh poultry samples ($n = 63$) vs. 77% of frozen samples ($n = 44$) indicated the reduction of *Campylobacter* spp. during the poultry freezing process. Lee et al. (1998) demonstrated that *C. jejuni* artificially inoculated onto chicken skin survives freezing under various atmospheric conditions. Their data did indicate a rapid reduction in survival during the first 2 weeks of −20 °C storage.

Human campylobacteriosis reached epidemic proportions in Iceland in 1999 as compared to 1997 (Stern et al., 2003). Levels of domestic origin had risen from less than 20/100,000 to 116/100,000 and the Public Health authorities were under considerable public pressure to reduce the problem. Fresh poultry was identified as the likely source for this upsurge in disease. In 2000, the Icelandic official veterinary authorities decided to employ freezing of poultry products as a mitigation strategy to reduce human exposure to *Campylobacter*. The central purpose of the current study was to quantify the effects of freezing and frozen storage on the numbers of *Campylobacter* and fecal coliforms in naturally contaminated broilers. As part of this study we compared two *Campylobacter* enumeration procedures, and assessed the effect of cold storage on the survival of the organism.

2. Materials and methods

All samples in these studies were obtained from two poultry processing plants in Iceland; following evisceration, processing plant A uses immersion chilling of broiler carcasses and processing plant B uses spray chilling. These represent two of the four poultry processing plants operated in Iceland and produce approximately 90% of the broiler meat consumed in the country.

2.1. Trial 1—Long-term, frozen carcass storage and sampling

A total of 90 moisture-impervious, plastic heat-sealed broiler carcasses were obtained from processing plant A. All the carcasses were from the same flock of broilers, harvested at 5–6 weeks of age, which had tested positive for *Campylobacter* at 4 weeks of age. All samples were gathered within $\frac{1}{2}$ h from the processing line. The weight of the sample carcasses ranged from 900 to 1300 g. Immediately after packaging, ten fresh carcasses were transported to the laboratory, stored at 2–4 °C and analysed within 24 h. The remaining 80 carcasses were frozen at the processing plant and then stored at −20 °C during the trial period. The frozen carcasses were divided into four groups containing 20 carcasses each. After storage times of 31, 73, 122 and 220 days, one group of carcasses was transported to the laboratory for microbiological analysis. At the laboratory, each group was divided into two groups of ten carcasses. One group was thawed at 7 °C for 20 h and the other group at 22 °C for 16 h before analysis.

Similar to the methods as described by Stern and Robach (2003), each individual carcass sample was weighed and aseptically placed into a sterile plastic bag. To each bag, 225 ml of sterile buffered peptone water (BPW; CM509:Oxoid) was added and each carcass vigorously shaken by hand for 60 s. After rinsing, the carcass was aseptically removed and the rinse BPW used for microbiological analysis.

2.2. Trial 2—Freezing and 31-day frozen storage

In trial 2, four additional 5–6-week-old flocks of *Campylobacter* positive broilers (as determined by