

EFFECT OF REFRIGERATION AND FROZEN STORAGE ON THE *CAMPYLOBACTER JEJUNI* RECOVERY FROM NATURALLY CONTAMINATED BROILER CARCASSES

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Submitted: March 16, 2009; Approved: November 07, 2009.

ABSTRACT

Campylobacter jejuni is the most common thermophilic *Campylobacter* associated with human enteritis in many countries. Broilers and their by-products are the main sources for human enteritis. Refrigeration and freezing are used to control bacterial growth in foods. The effect of these interventions on survival of *Campylobacter jejuni* is yet not quite understood. This study evaluated the effect of storage temperature on the survival of *C. jejuni* in chicken meat stored for seven days at 4°C and for 28 days at -20°C. The influence of selective enrichment on recovery of *Campylobacter* was also evaluated. Thirty fresh chicken meat samples were analyzed and 93.3% was contaminated with thermotolerant *Campylobacter* spp. with average count of 3.08 Log₁₀ CFU/g on direct plating. After refrigeration, 53.3% of the analyzed samples tested positive for *Campylobacter* and the average count was 1.19 Log₁₀ CFU/g. After storage at -20°C, 36.6% of the samples were positive with a average count of 0.75 Log₁₀ CFU/g. *C. jejuni* was detected after enrichment, respectively, in 50% of the fresh, 36.7% of the refrigerated and 33.3% of the frozen meat samples analyzed. No difference was detected for the recovery of *C. jejuni* from fresh, refrigerated or frozen samples after selective enrichment, showing that this microorganism can survive under the tested storage conditions.

Key words: *Campylobacter*, frozen storage, refrigeration, broiler meat.

INTRODUCTION

Campylobacter spp cause human bacterial gastroenteritis and are significant foodborne pathogens. *C. jejuni* is the most common species responsible for human illness. Although rarely fatal, *C. jejuni* infections are associated with post-infection complications including Guillian-Barré Syndrome, Miller Fisher Syndrome, reactive arthritis, Reiter's Syndrome and haemolytic uraemic syndrome (8, 21, 23, 27).

C. jejuni is part of the saprophyte intestinal flora of poultry, swine, and cattle and the epidemiological evidence

suggests that these animals may be reservoirs for strains that infect humans. Poultry and poultry products have been implicated as a major source of *Campylobacter* infection in humans (15). The prevalence of *Campylobacter* in raw poultry products ranges from 0 to 100% (6, 16). Although Brazil is the world's largest chicken exporter, little information has been published on the occurrence of *Campylobacter* spp. in Brazilian chicken products. Studies carried out on retail chicken in São Paulo State during 1986 and 1987 showed that 47.5% and 62.2%, respectively, of the analyzed samples were contaminated with *Campylobacter* spp. (1, 18).

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Refrigeration and freezing are used to control bacterial growth in foods. The effect of these interventions on survival of *C. jejuni* is not entirely clear. Different authors showed that counts of *C. jejuni* in poultry carcasses decrease during refrigeration or freezing (2, 30) and several studies demonstrated that *Campylobacter* survive in raw and cooked poultry meat during refrigerated or frozen storage (3, 4, 9, 17, 25, 26, 29). Many factors can cause injury or death of *Campylobacter* cells such as ice nucleation, dehydration or oxidative stress (24). The detection of viable cells after chilled or frozen storage is important as the *C. jejuni* infectious dose is thought to be about 500 cells.

The detection of thermotolerant *Campylobacter* in foods requires selective enrichment broths at 42°C under microaerophilic atmosphere (5) thus providing adequate conditions for growth of thermotolerant species, protection against toxic oxygen derivatives and recovery of injured cells (10).

This study evaluated the effect of refrigeration and freezing on survival of *C. jejuni* in chicken carcasses as well as the influence of selective enrichment on recovery of *C. jejuni*.

MATERIALS AND METHODS

Sample Preparation

Thirty broiler carcasses obtained from a commercial processing plant between September and November 2006 were analyzed. The carcasses were collected less than one hour after packaging. From each carcass three different 25-g aliquots were prepared with portions of skin from cloacae, neck, leg and drumstick. One of the 25-g aliquots was analyzed on the same day (fresh samples), the second one was analyzed after seven days at 4°C and the third one after 28 days at -20°C. These conditions were chosen to simulate the conditions to which poultry meat is exposed during processing and subsequent commercial storage. The 25-g aliquots were placed in Stomacher 400 filter bags containing 225 ml of buffered peptone water (HiMedia Laboratories, Mumbai, India). The samples and diluent were mixed in Stomacher 400 (Lab System Ltd, USA) for 60 seconds. The sample rinses were used

for microbiological analysis. For each experiment, 25-g samples of skin obtained from chicken carcasses not contaminated with *Campylobacter* spp. were spiked with approximately 100 CFU of *C. jejuni* ATCC 33291 (Adolfo Lutz Institute, São Paulo, Brazil) and used as positive control. Uncontaminated and uninoculated 25-g samples of chicken skin were used as negative controls. The microbiological analysis of the controls was performed as described below.

Thermotolerant *Campylobacter* spp count by direct plating

The modified Bolton agar described by Franchin; Aidoo and Batista (13) was prepared as follows: dehydrated Bolton broth (CM 983; Oxoid, Basingstoke, Hampshire, England) was suspended in water, added 1.5% agar-agar (HiMedia Laboratories, Mumbai, India) and 0.5 g/L ferrous sulphate (J. T. Baker, Germany). After adjusting to pH 7.5. and sterilization by autoclaving at 121°C for 15 min., the medium was cooled to 50°C and added aseptically 200 ppm of 2,3,5-triphenyltetrazolium chloride solution (TTC) (Sigma, USA) sterilized by membrane filtration and selective supplement (SR183E; Oxoid) containing 20 mg/L cefoperazone, 20 mg/L trimethoprim, 20 mg/L vancomycin and 50 mg/L cycloheximide. The medium was poured using 20 ml per Petri plate. Aliquots of 0.2 ml of chicken skin sample rinses were spread onto modified Bolton agar plates incubated at 37 °C for 4 h and then at 42 °C for an additional 44 h period under microaerophilic conditions (5% oxygen, 10% carbon dioxide, and 85% nitrogen) created with the Microarobac System (Probac do Brasil, São Paulo, São Paulo, Brazil). Identification of thermotolerant *Campylobacter* isolates was based on colony characteristics (magenta color, small, convex, shiny and with perfect edge), Gram stain and growth exclusively in a microaerophilic atmosphere. The *Campylobacter* colonies were counted and the results expressed as Colony Forming Units per gram of sample (CFU/g).

C. jejuni detection after selective Enrichment

Five-ml aliquots of sample rinses were enriched with 45 ml of Bolton broth (Merck) containing selective supplement (SR183E; Oxoid) at 37 °C for 4 h and then at 42 °C for an

additional 44 h period under microaerophilic conditions. After this incubation period a portion of enrichment broths was subcultured on Bolton modified agar plates. The plates were incubated at 42 °C for 24 hours under microaerophilic conditions. Identification of *Campylobacter* isolates were based on colony characteristics, Gram stain, motility under phase contrast microscopy (Bioval Model L2000A) and by the catalase test. Identification of *C. jejuni* was based on the hippurate hydrolysis test. The results were expressed as presence or absence of *C. jejuni* in 0.5 gram of sample.

Statistical Analysis

Nonparametric tests of Kolmogorov-Smirnov (19, 28) and Mann-Whitney (20) were used to evaluate thermotolerant

Campylobacter counts obtained from fresh, refrigerated and frozen chicken meat samples by direct plating. Case-Control Test by Chi-Square with 5% of probability (12) was used to analyze the influence of storage conditions and use of selective enrichment broths on the survival and recovery of *C. jejuni*.

RESULTS AND DISCUSSION

Average plate count results for thermotolerant *Campylobacter* spp after direct plating of the 30 poultry meat samples analyzed are presented in Table 1. A significant difference for plate counts at a level of 95% was obtained between results for fresh meat samples as compared to refrigerated and frozen samples.

Table 1. Number of positive samples and average plate count results for thermotolerant *Campylobacter* spp after direct plating.

Sample	Number of positive samples/ number of samples analyzed (%)	Average Log ₁₀ CFU/g
Fresh chicken meat ⁽¹⁾	28/30 (93.3) ^a	3.08 ^a
Chilled chicken meat ⁽²⁾	16/30 (53.3) ^b	1.19 ^b
Frozen chicken meat ⁽³⁾	11/30 (36.7) ^b	0.75 ^b

Different letter in the same column means a significant difference between samples (P<0.05)

⁽¹⁾ Samples analyzed one hour after chicken carcass packaging

⁽²⁾ Samples kept chilled at 4°C for 7 days

⁽³⁾ Samples kept frozen at -20°C for 28 days

Campylobacter was isolated from all skin chicken samples spiked with approximately 100 CFU of *C. jejuni* ATCC 33291 and it was not isolated from uncontaminated and uninoculated skin chicken samples.

The count reduction found in refrigerated and frozen samples was approximately 1.88 and 2.33 Log₁₀ CFU/g respectively. These results are higher than the ones obtained by Bhaduri & Cottrell (4) who found a reduction of 0.63 Log₁₀ CFU/g after direct plating of refrigerated chicken skin samples artificially contaminated with *C. jejuni* and a reduction of 1.38 to 2.26 Log₁₀ CFU/g for frozen samples. On the other hand, a

five time smaller incidence of *C. jejuni* was detected by Stern *et al.* (30) in frozen samples as compared to fresh meat samples. Likewise, Georgsson *et al.* (14) found a significant reduction of 0.65 to 2.87 Log₁₀ CFU/g for *Campylobacter* counts in samples stored at -20°C for 31 days.

Count reduction after direct plating or after selective enrichment of the chilled or frozen samples might be explained by death or cellular injury which induces *Campylobacter* cells to assume the “viable but not cultivable” (VNC) form. This form can not be recovered by conventional methods (22).

C. jejuni identification was made only after selective

enrichment and no significant difference ($P > 0.05$) was found for *C. jejuni* detection rate from fresh samples or those submitted to low temperatures. Similar results were obtained by Meldrum *et al.* (22). However, Alter *et al.* (2) detected a significant reduction rate for *C. jejuni* detection (from 67.4 to 25.6%) in 43 turkey meat samples that were held at 0°C to 3°C for 24 hours.

Lee *et al.* (17) showed that *C. jejuni* was able to survive for up to 56 days under frozen storage at -20°C, which is the temperature found in most domestic freezers. According to these authors, *C. jejuni* was viable even after a count reduction of approximately 5 Log₁₀. Georgsson *et al.* (14) also demonstrated that in spite of a significant *Campylobacter* count reduction in chicken meat stored at -20°C for 31 days, the counts remained at approximately 3.0 Log₁₀ CFU/g. Thus, frozen storage of foods can not be considered a safety assuring procedure when the infection dose for *Campylobacter* is thought to be about 500 cells.

Fernández and Pisón (11) found 117 (92.9%) positive results for *Campylobacter* spp in 126 chicken liver samples analyzed through a selective enrichment method. From these positive samples, 92 (73.0%) were identified as *C. coli* and 25 (19.8%) as *C. jejuni*. The authors suggested that *C. coli* could be more resistant to injury resulting from exposure to low temperatures and adverse ambient conditions.

In two samples analyzed in the present work, *Campylobacter* was detected after direct plating in fresh but not in chilled and frozen samples. However after selective enrichment *C. jejuni* was isolated as much in fresh as in samples kept under refrigerated or frozen storage. Similar results were obtained with two other refrigerated and two frozen samples, where *C. jejuni* was detected only after enrichment.

According to Bolton and Robertson (5) the direct plating technique can be employed in clinical diagnosis laboratories where *Campylobacter* counts in feces are found to be high, while for studies which are based on low *Campylobacter* counts, such as in food samples, the enrichment method should be used. The results found in present work showed that the

direct plate should be done in parallel with the enrichment method for detection of *Campylobacter*, specially from refrigerated or frozen foods.

Iceland decided in 2000 to adopt frozen storage of chicken meat as a means to reduce human exposure to *Campylobacter*. Norway adopted the same strategy in 2001 (14). Nevertheless the results obtained in the present work show that when research is done with selective enrichment, the *C. jejuni* detection rate in fresh, chilled or frozen chicken meat samples are not significantly different, demonstrating that quite a number of *C. jejuni* cells survive low temperatures. This confirms that chilling or frozen procedures do not assure food safety when concerning this particular microorganism.

The lack of recovery of injured cells by directing plating might be the explanation of the failure of *Campylobacter* detection or the significant lowering of counts in the refrigerated and frozen chicken meat samples analyzed in this research. However with the selective enrichment technique no significant differences were found for *C. jejuni* detection rate between fresh chicken meat samples and those kept under refrigerated (4°C) storage for 7 days or those kept under frozen (-20°C) storage for 28 days, clearly demonstrating that this organism survives these adverse conditions.

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