Research Note

Survival of *Campylobacter jejuni* on Poultry Skin and Meat at Varying Temperatures

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ABSTRACT Researchers have recently found a much higher prevalence of *Campylobacter* on skin-on poultry products vs. skinless products. These data suggest that contamination is associated primarily with poultry skin, and *Campylobacter* may not survive on skinless poultry meat. Therefore, the objective of this study was to quantify the survival of *Campylobacter* poultry skin vs. meat under differing storage conditions. Skin and meat were irradiated to eliminate native microflora and inoculated with *Campylobacter jejuni* (~5.0×10^5 cfu/mL). Meat and skin samples were packaged in polystyrene trays, covered with Cryovac film, and then subjected to 1 of the following storage conditions: 1) 4°C for 11 d; 2) 4°C for 1 d, then –3°C for 10 d; 3) 4°C for 1 d, –3°C for 1 d, then 4°C for 9 d; or 4) 4°C for 1 d, –3°C for 1 d, 20°C for 1 h on d2, then 4° for 9 d. On d 0, 2, 3, 5, 7, 9, and 11, populations of *Campylobacter* were determined. The experiment was replicated 3 times. In each experiment, populations of surviving *Campylobacter* were not affected by storage conditions ($P \geq 0.05$), and there was no interaction between temperature treatments and sample type. Surviving *Campylobacter* populations were affected ($P \leq 0.05$) by sample type (skin vs. meat). *Campylobacter*, in the absence of competing microflora, survived well on poultry skin and meat at the temperatures tested. In all experiments, higher populations were established on the inoculated skin vs. inoculated meat. These populations remained consistently 0.4 to 0.9 $\log_{10}$ cfu/g higher on skin vs. meat. Poultry skin topography may account, in part, for these higher populations on skin.

Key words: *Campylobacter jejuni*, poultry skin, poultry meat

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INTRODUCTION

Campylobacter jejuni is a part of the thermotolerant group of the family *Campylobacteriaceae* and is considered to be a human pathogen of foodborne origin. According to a baseline study conducted by the USDA Food Safety and Inspection Service in 1994 to 1995, the prevalence of *Campylobacter* on immersion-chilled poultry carcasses is 88.2% (Food Safety and Inspection Service, 1996). Most outbreaks of *C. jejuni* occur in the spring and fall and are attributed to either the consumption of raw, unpasteurized milk or contaminated water (ICMSF, 2002). However, sporadic cases during the summer months are mainly attributed to handling or consumption of undercooked poultry (ICMSF, 2002). The US Centers for Disease Control and Prevention surveillance has identified *C. jejuni* as one of the leading causes of diarrheal disease in the United States (Centers for Disease Control and Prevention, 1999, 2001). Enteritis caused by *Campylobacter* manifests itself much like other intestinal infections caused by bacteria. It is very similar to salmonellosis and shigellosis (Conner et al., 2001). It is known that *Campylobacter* can survive in the environment, especially in untreated surface water (Nachamkin and Blaser, 2000), but it is primarily transferred onto poultry carcasses via fluid and feces from the gastrointestinal tract of the bird, due to the high numbers of the organism found in these fluids (Franco and Williams, 2001). The organism then attaches to the skin of the broiler and perseveres to final products (Benefield, 1997). The majority of viable *Campylobacter* cells found on poultry skin have been trapped in either the surface water layer or entrapped with water in skin crevices or feather follicles (Chantarapanont et al., 2003). These data suggest that poultry skin is a safe harbor for *Campylobacter* species and may explain why Davis and Conner (2000) reported that the incidence on raw, retail poultry products decreases from 76% on whole broilers to 48% on skin-on split breast to only 2% on boneless, skinless breast meat.

Because most of the intermittent cases of human campylobacteriosis are attributed to mishandling or improper preparation of poultry (Bryan and Doyle, 1995; ICMSF, 2002) and previous studies by these researchers have shown that the incidence of contamination of raw, retail poultry products varied greatly, this study was con-

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Table 1. Storage conditions and temperature schemes for poultry skin and meat samples

Storage condition	Temperature scheme followed	Simulation
1	4°C for 11 d	Normal refrigeration temperature
2	4°C for 1 d –3°C for 10 d	Normal refrigeration, then simulated surface ice crusting
3	4°C for 1 d –3°C for 1 d 4°C for 9 d	Standard refrigeration, followed by ice crusting, then normal refrigeration
4	4°C for 1 d –3°C for 1 d 20°C for 1 h (d 2) 4°C for 9 d	Simulates normal refrigeration, followed by ice crusting, then temperature abuse, and normal refrigeration

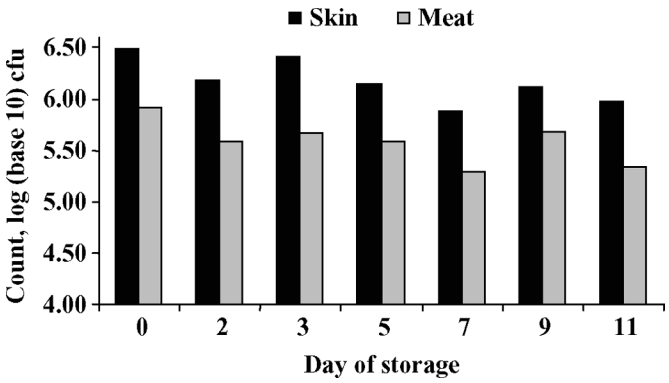

Figure 1. Numerical comparison of average populations of *Campylobacter jejuni* on inoculated poultry skin vs. meat at different days of storage, all trials.

ducted to determine how differing temperatures, storage conditions, and skin covering would affect the survival of *C. jejuni.*

MATERIALS AND METHODS

In each trial, skin pieces were cut to a diameter of 10 cm. Meat pieces were approximately 16 cm^3 to provide similar surface areas for bacterial attachment. Meat and skin samples were irradiated to 10 kGy using cobalt 60 to eliminate background microflora. Each of the sample pieces was then individually inoculated (1.0 mL) with a known isolate of *C. jejuni* (~5.0×10^5 cfu/mL). The inoculum was a wild strain of *C. jejuni* obtained from commercial poultry. The inoculum culture was grown in brain heart infusion broth under microaerophilic conditions for 48 h at 42°C. Samples were then placed into 0.45-kg capacity styrofoam trays, obtained from the Auburn University Lambert Meats Lab, and covered with aerobic Cryovac film, obtained from Sealed Air Corporation, Saddle Brook, New Jersey. Each of the trays contained 4 samples of either meat or skin that were used as sample units for analysis. Sample units for each day were 16 each of skin and meat (4 samples × 4 storage conditions). Equal numbers of samples were subjected to 1 of 4 temperature conditions (Table 1). The storage conditions used imitated differing refrigeration schemes that processed poultry might be exposed to. These included normal refrigeration, normal refrigeration followed by surface ice crusting, normal refrigeration followed by ice crusting and back to normal refrigeration, and finally normal refrigeration followed by surface ice crusting then possible temperature abuse and back to normal refrigeration.

Populations of *C. jejuni* on meat and skin samples were enumerated on d 0, 2, 3, 5, 7, 9, and 11. At these times, individual samples were placed in Whirl-Pak bags (Nasco, Fort Atkinson, WI) and a 1:10 dilution by weight was made using 0.1 *M* K phosphate buffer. This mixture was then stomached for 1 min. Serial dilutions were made from the original 1:10 dilution and were spiral-plated onto Campy-Cefex agar with a WASP spiral plater (Microbiology Int., Frederick, MD). The plates were incubated in a microaerophilic environment for 48 h at 42°C. Plates were counted using a laser counter programmed to read spiral plates (bacteria colony counter, model 500A, Spiral Systems Inc., Bethesda, MD). Initial mean *C. jejuni* populations ($\log_{10}$ cfu/mL) were as follows: trial 1, 7.94; trial 2, 7.75; and trial 3, 7.77. Surviving populations were converted to logarithm base 10 values and subjected to statistical analysis using the PROC GLM, with Tukey's studentized range, and LSMEANS statements of SAS (SAS Institute, 1997).

RESULTS AND DISCUSSION

In each of the 3 trials, the temperature condition in which the samples were held (Table 1) had no effect ($P > 0.05$) on the number of *Campylobacter* that survived (Table 2). This is consistent with survival studies conducted in Norway, in which thermotolerant species of *Campylobacter* survived well at 4°C (Franco and Williams, 2001). *Campylobacter* also can be cultured from frozen poultry meat (Nachamkin and Blaser, 2000). However, the type of sample that was inoculated (meat vs. skin) significantly affected *Campylobacter* survival. This observation suggests that the organism has an affinity for the skin over skinless product, most likely due to the niche environments provided by skin crevices and feather follicles (Chantarapanont et al., 2003). The affinity that the organism has for these environments could be due to the availability of water as a medium and lowered ability of the organism to come into contact with O_2. Storage time also significantly affected the amount of *Campylobacter* that survived (Table 2). Populations were highest for each trial on d 0 and generally declined until d 7. There were no significant interactions among the experimental values (Table 2). Because differing storage conditions did not affect *C. jejuni* populations, a comparison was made between meat and skin at each sample day (Figure 1). In all 3 trials, higher populations were found on skin vs. meat on all sample days. This further indicates that the organism may have an affinity for poultry skin that is not fully understood.

Although populations were higher on poultry skin than meat, introduced *Campylobacter* survived well on both

Table 2. Factorial design for 3-way interaction among storage condition, sample type, and sample day

Item	Trial 1 mean *Campylobacter jejuni* population (log_{10} cfu/mL)	Trial 2 mean *C. jejuni* population (log_{10} cfu/mL)	Trial 3 mean *C. jejuni* population (log_{10} cfu/mL)
Storage condition (T)	NS[1] (0.5793)	NS (0.2707)	NS (0.0782)
1	6.11	5.57	5.75
2	6.13	5.55	5.47
3	6.16	5.57	5.63
4	6.05	5.49	5.17
Sample type (P)	***	***	***
Meat	5.78[b]	5.30[b]	5.21[b]
Skin	6.44[a]	5.79[a]	5.80[a]
Sample day (D)	***	***	*
0	6.51[a]	5.92[a]	6.02[a]
2	6.08[b]	5.62[bc]	5.62[ab]
3	6.55[a]	5.54[bc]	5.75[ab]
5	6.05[b]	5.78[ab]	5.05[b]
7	6.09[b]	4.83[d]	5.58[ab]
9	6.00[b]	5.63[bc]	5.19[ab]
11	5.51[c]	5.48[c]	5.33[ab]
Interactions			
T × P	NS (0.1288)	NS (0.8273)	NS (0.9541)
T × D	NS (0.7545)	NS (0.1003)	NS (0.9791)
P × D	NS (0.2836)	NS (0.0753)	NS (0.0789)
T × P × D	NS (0.4284)	NS (0.9063)	NS (0.3459)

[a–d]Differing superscripts within the main effect column indicate significant differences.

[1]Not significant ($P > 0.05$).

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

skin and meat at varying temperatures in the absence of competing microflora. These data suggest that if the organism is introduced at levels that are found in poultry fecal material, it can survive in numbers large enough to be considered an infective dose (~500 cfu) for humans (Nachamkin and Blaser, 2000). Because this study was conducted in the absence of competing microflora, it is also possible that other organisms affect the populations of *Campylobacter* on poultry products. Mai (2003) concluded that many organisms associated with poultry products in the processing environment, such as the pseudomonads, micrococci and staphylococci, inhibit the growth of *C. jejuni.* These organisms inhibited growth in a range from log_{10} 1.05 to 5.77. These findings, along with the findings in this study, suggest that a complex mechanism determines the extent of survivability of *Campylobacter* on poultry products.

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