ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 3 (7): 432-437, 2004

Spread of *Campylobacter* spp. During Poultry Processing in Different Seasons

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Abstract: The presence of *Campylobacter* spp. on broiler carcasses and in scald tank water in a commercial poultry processing facility was monitored at monthly intervals from July through December. The spread of the pathogen had previously been monitored in the same facility from January through June of the same year. *Campylobacter* were enumerated on prescalded, picked, eviscerated, and chilled broiler carcasses; on processed carcasses stored at 4°C for 7 or 14 days, and in scald tank water samples. The fatty acid methyl ester (FAME) profile of the *Campylobacter* isolates and the degree of relatedness between the *Campylobacter* isolates was determined using the MIDI Sherlock Microbial Identification System (MIS). Findings indicated that *Campylobacter jejuni* was present on carcasses and in scald tank water samples collected from July through December. Processing significantly ($P < 0.05$) decreased the number of *Campylobacter* recovered from broiler carcasses, however. Furthermore, significantly ($P < 0.05$) fewer *C. jejuni* were consistently recovered from the third tank of the multiple tank scald system than from the first tank. Findings indicated that poultry flocks may introduce several strains of *C. jejuni* into processing facilities. Additionally, different populations of the pathogen may be carried into the processing plant by successive broiler flocks, and some strains of *C. jejuni* may reappear in the same processing facility during different times of the year.

Key words: *Campylobacter*, poultry processing, broilers

Introduction

Campylobacter spp. are common contaminants of live broilers and their environment (Genigeorgis *et al*., 1986). Commercial broiler processing operations such as scalding, defeathering (picking), evisceration, and chilling may affect the level of carcass contamination by foodborne pathogens and spoilage microorganisms. Although most processing steps reduce microbial contamination of broilers, the potential for processing operations to spread carcass contamination to processing equipment, water, other carcasses, and processing personnel has also been reported (Stern *et al*., 2001). Processed poultry products contaminated with the bacterium may then serve as vehicles for campylobacteriosis outbreaks in humans (Bryan and Doyle, 1995). *Campylobacter jejuni* and *Campylobacter coli* are the species of the pathogen that are most frequently isolated from poultry (Hudson *et al*., 1999).

Campylobacter and other microorganisms may be identified based on their cellular fatty acid profile (Glucksman *et al*., 2000). Automated systems, such as the MIDI Sherlock Microbial Identification System (MIS, 2002), can identify bacteria, yeasts, and fungi by comparing the fatty acid methyl ester (FAME) profiles of unknown microorganisms to the profiles of microorganisms stored in the system's library (Leonard *et al*., 1995). The MIS software also contains a Dendrogram Program that can be used to determine the degree of relatedness of microbial isolates identified by the system. Microorganisms that are closely related and belong to the same microbial strain are considered to have the same source of origin. Consequently, by determining which processing operations are contaminated by the same bacterial strain, the movement of bacteria through processing facilities may be determined.

The purpose of the present study was to complete the year-long analysis of the movement of *Campylobacter* spp. through a commercial poultry processing facility and to examine seasonal variation of the level of *Campylobacter* contamination of broiler chickens. *Campylobacter* have been reported to exhibit a cyclical pattern of contamination of poultry (Willis and Murray, 1997), whereas, the level of contamination consistently

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increase and decrease depending on the season of the year. An earlier study examined the presence of *Campylobacter* during processing from January through June. To determine if seasonal changes were reflected in the movement of Campylobacter through poultry processing facilities, the present study examined the presence of the organism in the same facility from July through December. Findings from these studies may identify those processing steps that facilitate or reduce the spread of *Campylobacter* during processing operations. Such information may be useful in developing strategies for decreasing the number of cases of human campylobacteriosis associated with contaminated poultry.

Materials and Methods

Samples were taken from a commercial poultry processing plant at monthly intervals from July through December. Six each of prescalded (PS), picked (PP), and eviscerated (EV) carcasses, and 18 chilled (CH) carcasses were collected from the processing facility during each visit, as previously described (Hinton *et al.*, 2004a). Six water samples were also taken from each of the 3 tanks of the facility's counter flow scald tank system. Carcasses and scald tank water samples were transported immediately to the laboratory. All samples were subjected to microbial analysis upon arrival at the laboratory, except for 12 carcasses that were stored at 4°C and analyzed after 7 (R7) or 14 (R14) days of storage.

Campylobacter from carcasses and scald water were enumerated by direct plating, as previously described (Hinton *et al.*, 2004a). Serial dilutions of samples of whole carcass reinstates and scald tank water were prepared in 0.1% Bacto Peptone (Difco) solutions and plated on Bacto *Campylobacter* Agar, Blaser (Difco) plates. Inoculated plates were incubated at 42°C for 48 h under microaerobic conditions and examined for the presence of typical *Campylobacter*-like colonies. Enumeration procedures were unable to detect less than log 1.00 colony-forming-units (cfu) of *Campylobacter*/ml, and a value of $\log_{10}$ 0.99 cfu/ml was assigned to samples from which no *Campylobacter* were recovered by direct plating. Selected colonies were taken from the incubated plates and subjected to the Latex-CAMPY(jcl)™ *Campylobacter* Culture Confirmation Test, Latex-CAMPY (jcl) ™ (Integrated Diagnostics, Inc. Baltimore, MD 21227). The identity of colonies that were positive for the *Campylobacter* latex agglutination test was confirmed using the automated MIDI Sherlock Microbial Identification System (MIS), Version 4.5 (MIDI, Inc., Newark, DE 19713) (Hinton *et al.*, 2004a).

After enumeration of *Campylobacter* in the processing samples, the GraphPad InStat version 3.05, 32 Bit for Windows 95/NT (GraphPad Software, San Diego California USA) was used for statistical analysis of data. A One-Way Analysis of Variance (ANOVA) was conducted to determine if there was a significant difference between group means. The Tukey-Kramer Multiple Comparisons Test was used to determine which treatment groups differed significantly when the ANOVA detected significant differences in group means. All significant differences were determined at $P < 0.05$.

The Dendrogram Program of the MIDI MIS was used to establish the degree of relatedness between *Campylobacter* isolates. The Dendrogram Program determines the relatedness of microorganisms based on the Euclidean Distance (ED) that separates the FAME profiles of the isolates. (LGS User's Manual, 2002). Microbial isolates linked at an ED of $\leq$ 10.0 generally belong to the same species; isolates linked at an ED of $\leq$ 6.0 generally belong to the same subspecies; and isolates linked at an ED of $\leq$ 2.5 generally are considered to be the same microbial strain (Leonard *et al.*, 1995; LGS User's Manual, 2002).

Results and Discussion

Campylobacter were recovered from broiler carcasses and scald tank water samples taken from the processing facility in every month from July through December (Table 1 and 2). During a 12-month period, the level of contamination of broiler chickens by *Campylobacter* generally exhibits a cyclical pattern (Hudson *et al.*, 1999; Willis and Murray, 1997). The cycle of contamination consists of an increase in the amount of *Campylobacter* recovered from broilers and their environment during warmer months, and a decrease in the amount of these bacteria recovered during colder months. The continued recovery of *Campylobacter* during the late fall and winter in the present study may have been related to the mild temperatures that the broiler growing area experienced during the year that the samples were collected. The average high/low temperatures for the months when *Campylobacter* populations are expected to begin to decline were 28°/17°C for September, 23°/10°C for October, 18°/6°C for November and 13°/2°C for December (Weather Underground, 2004). In the earlier study in which no *Campylobacter* was recovered from samples taken from the plant in January and February (Hinton *et al.*, 2004a), the average high/low environmental temperatures were 11°/0°C and 14/2°C, respectively. During March through June, when *Campylobacter* were first recovered from samples taken from the facility, the low temperature was never less than 6°C and the high temperature reached as high as 31°C. The exposure of broilers to lower temperatures for extended periods of time may be a factor in decreasing *Campylobacter* populations in broiler flocks during cooler Fall and Winter months.

As seen in an earlier study (Hinton *et al.*, 2004a), processing operations in the current study significantly ($p < 0.05$) reduced the level of contamination of broiler

Table 1: Log_{10} colony-forming-units (cfu)/ml and *Campylobacter*-positive samples/total samples recovered on *Campylobacter*, Blaser agar from reinstates of broiler carcasses collected from various stages of commercial processing during July through December

	Month[1, 2]		
Broiler Carcass	July	August	September
Prescalded	4.13[a] ± 1.13 (6/6)	3.68[a] ± 1.28 (6/6)	5.40[a] ± 0.19 (6/6)
Picked	1.04[b] ± 0.13 (1/6)	3.31[a] ± 0.50 (6/6)	3.93[b] ± 0.44 (6/6)
Eviscerated	<1.00[b] (0/6)	1.64[b] ± 0.25 (6/6)	2.95[c] ± 0.37 (6/6)
Chilled	<1.00[b] (0/6)	0.99[b] ± 0.01 (1/6)	1.67[d] ± 0.42 (5/6)
Refrigerated 7 days	<1.00[b] (0/6)	<1.00[b] (0/6)	<1.00[e] (0/6)
Refrigerated 14 days	<1.00[b] (0/6)	<1.00[b] (0/6)	<1.00[e] (0/6)
	October	November	December
Prescalded	3.23[ab]± 1.74 (4/6)	5.08[a] ± 0.25 (6/6)	4.62[a] ± 0.55 (6/6)
Picked	3.61[a] ± 0.75 (6/6)	3.99[b] ± 0.86 (6/6)	1.58b ± 1.44(1/6)
Eviscerated	2.74[ab] ± 0.44 (6/6)	1.93[c] ± 0.76 (4/6)	<1.00[b] (0/6)
Chilled	2.02[bc] ± 0.34 (6/6)	1.43[cd] ± 0.33 (5/6)	1.29[b] ± 0.53 (2/6)
Refrigerated 7 days	1.13[c] ± 0.21 (4/6)	1.11[cd] ± 0.29 (1/6)	1.37[b] ± 0.42 (2/6)
Refrigerated 14 days	1.01[c] ± 0.25 (3/6)	<1.00[d] (0/6)	<1.00[b] (0/6)

[1]Values averages ± standard deviation log10 cfu/ml (positive samples/total samples)

[2]Values < 1.00 indicate that no *Campylobacter* were recovered by direct plating

[a-e] Within columns, different superscripts indicate significant differences in the number of cfu/ml recovered from whole carcass reinstates of the carcasses

Table 2: Log10 colony-forming-units (cfu)/ml recovered on *Campylobacter*, Blaser agar and *Campylobacter* positive samples/total samples collected from a commercial multiple-tank counterflow scald tank system

	Month[1,2]					
Scald Tank #	July	August	September	October	November	December
1	2.89[a]±0.14 (6/6)	5.61[a]±0.17 (6/6)	4.41[a]±0.29 (6/6)	5.40[a]±0.25 (6/6)	5.56[a]±0.50 (6/6)	3.68[a]±2.13 (4/6)
2	1.04[b]±0.13 (2/6)	4.31[b]±0.35 (6/6)	1.83[b]±1.31 (2/ 6)	2.70[b]±1.28 (2/6)	3.38[b]±0.26 (6/6)	1.83[a]±1.31 (2/6)
3	<1.00[b]± (0/6)	2.54[c]±0.24 (6/6)	<1.00[b]±(0/6)	<1.00[c] (0/6)	<1.00[c] (0/6)	<1.00[b] (0/6)

[1]Values averages ± standard deviation log10 cfu/ml (positive samples/total samples)

[2]Values of <1.00 indicate that no *Campylobacter* were recovered by direct plating

[a-c] Within columns, different superscripts indicate significant differences in the number of cfu/ml recovered from whole carcass reinstates

carcasses by *Campylobacter* (Table 1). The combination of scalding and picking operations decreased the number of *Campylobacter* recovered from carcasses in July, September, November, and December. Scalding may reduce carcass contamination because scald water can remove contaminated dirt and debris from feathers, feet, and skin of the broilers (Cason *et al.*, 1997) and because scald water temperatures of may be lethal to the bacteria (Yang *et al.*, 2001). Furthermore, the multiple tank counterflow scald system exposes carcasses to progressively cleaner water with progressively higher temperatures (Cason *et al.*, 2000) as the carcasses are moved through tanks #1, #2, and #3 with average water temperatures of 45.0, 49.9, and 57.2°C, respectively. Immersion scalding of carcasses in 58°C or 60°C water has been reported to reduce the number of *Campylobacter* on carcasses (Oosterom *et al.*, 1983). Significantly fewer *Campylobacter* were recovered from tank #2 than from tank #1 in July through November, and significantly fewer *Campylobacter* were recovered from tank #3 than from tank #2 in August, October, November, and December (Table 2). The reduction in the number of *Campylobacter* recovered from successive tanks of the multiple-tank counterflow scald tank system was also probably due to the progressively cleaner water in the tanks and to the progressive increase in the temperature of water in the tanks (Cason *et al.*, 2000) After scalding, broilers were immediately passed through a mechanical picker. Although feather picking operations can also reduce carcass contamination by physically removing microorganisms carried on feathers and skin of broilers, defeathering may potentially spread microorganisms among carcasses as they pass thorough the same mechanical picker (Berrang *et al.*, 2001). The combination of scalding and picking reduced the

Table 3: *Campylobacter jejuni* strains recovered from two or more samples collected in the same month (July through December)

Month Sample Collected	Strain No.	Processing operations[a] contaminated with the same strains
July	1	PS:1/PS:3
July	2	PS:4/PS:6/SC1:6
July	3	PS:5/PS:6/SC1:6
July	4	SC1:3/SC1:5
July	5	SC1:2/SC1:4/SC1:5
August	6	PS:4/ SC2:3
August	7	SC1:3/SC1:4
August	8	SC1:1/SC1:4/ SC1:5
September	9	PP:8/PP:10
September	10	PP:9/EV:13
September	11	PP:11/EV:15
September	12	PP:12/EV:13
September	13	EV:18/CH:24
September	14	CH:19/CH:21
October	15	PS:5/CH:20
October	16	SC1:3/SC1:5
October	17	SC2:4/SC2:5
October	18	SC2:6/EV:14/CH:23/R7:29
October	19	PP:7/CH:19
October	20	EV:13-EV:18
October	21	CH:23/R14:34
October	22	R14:34/R14:35
November	23	PS:3/PS:4
November	24	PS:6/PP:9
November	25	PS:7/PP:12
November	26	PS:2/SC2:5
November	27	SC2:1/SC2:3/SC2:4
November	28	SC1:2/PP:11
November	29	SC1:4/PP:8
November	30	SC2:4/R7:30
November	31	EV:16/CH:19
December	32	PS:5/PS:6/CH:19
December	33	PS:6/SC1:2/CH:24
December	34	PS:1/SC2:3
December	35	PS:1/PP:11
December	36	PS:4/PP:11
December	37	SC2:2/CH:19

[a]Processing sample (PS-prescalded carcass, PP-scalded and picked carcass, EV-eviscerated carcass, CH-chilled carcass, R7- carcass refrigerated for 7 days, R14-carcass refrigerated for 14 days, SC1-scald tank #1 water, SC2-scald tank #2 water, SC3-scald tank #3 water):and carcass or water sample number (1-6)

number of *Campylobacter* recovered from carcasses reinstates by over $\log_{10}$ 3.0 cfu/ml in July and December; however, in October $\log_{10}$ 0.38 cfu/ml more *Campylobacter* were recovered from reinstates of prescalded carcasses than from scalded and picked carcasses.

Other processing operations also reduced carcass contamination by *Campylobacter*. Evisceration significantly reduced the number of *Campylobacter* on carcasses processed in August, September, and November. The removal of the alimentary tract by evisceration eliminates a major source for carcass contamination by *Campylobacter* and other enteropathogens in poultry. Evisceration may also increase carcass contamination by *Campylobacter*, if contaminated intestinal contents are spilled onto the surface of the carcass during the process (Berrang *et al.*, 2001). There was no indication of increased *Campylobacter* contamination of carcasses due to evisceration in the present study; however, broilers processed during October, the first significant reduction in the number of *Campylobacter* was seen when chilling caused a significant reduction in the *Campylobacter* population that was found on picked carcasses. Chilling also significantly reduced *Campylobacter* contamination in broilers processed in September. Commercial processors usually add antimicrobial chemicals, such as chlorine, to the chill water to kill pathogenic and spoilage microorganisms on the carcass (Mead *et al.*, 1995). The presence of the antimicrobial chemicals in chiller water and the cleansing activity of the water may have played roles in reducing contamination of the carcasses processed in September. A second significant reduction in the *Campylobacter* contamination of broilers processed in September occurred after 7 days of refrigerated storage. The decrease of the *Campylobacter* population during storage may have been due to the sensitivity of the bacterium to conditions experienced during long term refrigerated storage, but there was no significant difference in the number of *Campylobacter* recovered from carcasses stored for 7 or 14 days.

Sherlock MIS FAME analysis identified numerous *C. jejuni* isolates that were recovered from the processing samples. An earlier study also recovered *C. coli* from broilers processed at this facility, but no *C. coli* were recovered from carcasses or water samples taken from the facility from July through December. After the isolates were identified by the Sherlock MIS, dendrograms were constructed (dendrograms not shown) to determine which isolates belonged to the same *C. jejuni* strain because they had FAME profiles that were separated by less than 2.5 ED.

Recovery of the same *C. jejuni* strain from different processing locations indicated that a group of bacteria with the same source of origin was present in samples taken from different carcasses or scald tanks and/or that flocks processed at the facility in different months were contaminated with the same *C. jejuni* strain. During July, 5 different *C. jejuni* strains were recovered that were isolated from two or more carcasses or scald tank water samples (Table 3). Strain #1 was recovered from two

Table 4: *Campylobacter jejuni* strains recovered from two or more samples collected in different months (July through December)

Strain number	Processing Samples Containing Same *C. jejuni* strainc
1[a]	Jul:PS:1/Jul:PS:3/ Oct:SC2:4/Oct:SC2:5/Dec:PS1:4
21[a]	Jul:SC1:6/Oct:CH:23/Oct:R14:34/Nov:SC1:1
38[b]	Jul:PS:1/Nov:SC1:6
39[b]	Jul:PS:3/Oct:SC2:6
40[b]	Sep:PS:6/Oct:SC1:5

[a]Strain listed in Table 3. [b]Strain not listed in Table 3. [c]Processing Sample-Month collected (July, August, September, October, November, December): Source (PS-prescalded carcass, PP-scalded and picked carcass, EV-eviscerated carcass, CH-chilled carcass, R7-carcass refrigerated for 7 days, R14-carcass refrigerated for 14 days, SC1-scald tank #1 water, SC2-scald tank #2 water, SC3-scald tank #3 water): and carcass or water sample number (1-6)

different prescalded carcasses, strains # 2 and 3 were recovered from two different prescalded carcasses and from scald tank #1, strain #4 was recovered from 2 scald tank #1 water samples, and strain #5 was recovered from three other samples taken from scald tank #1.

Evidence of cross contamination in samples collected in August also indicated that the same *C. jejuni* strain could be recovered from prescalded carcasses and from scald water that the carcasses had been passed through. Analysis of samples collected from September through December indicated that the same *C. jejuni* strain could also be recovered from picked, eviscerated, and refrigerated carcasses.

Unlike findings from the previous study (Hinton *et al.* 2004a), results of the present study indicated that the same *C. jejuni* strain could be found in samples collected in different months from the same processing facility (Table 4). *C. jejuni* strain #1 was recovered from prescalded broilers processed in July, from a scald tank #1 water sample collected in October, and from a prescalded carcass processed in December; while strain #20 was recovered from samples collected in July, October, and November. Two other strains isolated from the July flock were also recovered in different months. The grower who provided the broilers is not known, but it is possible that broilers processed in July were reared in the same location as some of the broilers processed in later months. Due to the fragility of the *Campylobacter* bacterium, it is unlikely that this microorganism colonized the processing facility, as some other more resistant microorganisms (Hinton *et al.*, 2002; Hinton *et al.*, 2004b), and was able to contaminate successive flocks after they arrived at the facility.

To reduce the number of cases of human campylobacteriosis associated with contaminated poultry products, processing operations must consistently reduce campylobacter contamination of carcasses to safe levels. Although current processing operations can decrease the *Campylobacter* contamination of processed carcasses, improvements in the ability of poultry processing operations to destroy *Campylobacter* spp. are still required to produce carcasses that are free of this major foodborne pathogen.

Acknowledgements

The authors acknowledge the technical assistance of Jerrie Barnett, Charles Hernandez, Fredda J. Murray, and Kathy Orr.

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