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Prevalence of *Campylobacter jejuni* in Two California Chicken Processing Plants

JOHN M. WEMPE, CONSTANTIN A. GENIGEORGIS,* THOMAS B. FARVER, AND HAROUN I. YUSUFU

Department of Epidemiology and Preventive Medicine, School of Veterinary Medicine, University of California, Davis, California 95616

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Two federally inspected California chicken processing plants participated in *Campylobacter jejuni* prevalence studies. Twelve sampling sites were included in each of four groups. Groups were based on bird age, scald water temperature, and plant sampled. Scald water temperatures of 60°C (140°F) did not contribute to a lower prevalence of *C. jejuni* in edible parts, as did temperatures of 53°C (127°F) and 49°C (120°F). The feather picker and chilling tank were areas of major cross-contamination. *C. jejuni* was isolated from 68% of the ready-for-market products. The organism was recovered from 60 to 100% of the ceca in the four groups, and some numbers in the fecal material exceeded 10^6/g. The level of *C. jejuni* in intestinal tracts seemed to correlate with the presence of the organism in the edible parts.

Campylobacter jejuni has long been recognized as a pathogen in animals, but recently it has been established as an important cause of severe diarrhea in humans (2, 3, 4, 7, 20). *C. jejuni* has a worldwide distribution and is as commonly associated with human diarrhea as are *Salmonella* spp. and *Shigella* spp. (1, 3, 6). Studies in Europe, North America, and Australia have shown *C. jejuni* to be the causative agent of diarrhea in 3 to 14% of patients (2). Extensive reports have been published on *C. jejuni* as a cause of human enteritis (3, 4, 7, 16), and foodborne (milk, poultry, pork, beef) transmission has been implicated as one route of infection (1, 7). Recent studies have revealed isolation rates for *C. jejuni* from processed poultry that range from 1.7 to 83% (5, 9, 13, 14, 17, 19, 22; H. Kinde, Master Preventive Veterinary Medicine report, University of California, Davis, 1981; H. M. Rayes, Master Preventive Veterinary Medicine report, University of California, Davis, 1982). Prevalence of the organism in the feces of chickens, turkeys, migratory waterfowl, and other birds ranges from 30 to 100% (9, 12, 19). Little information is available on the prevalence of the organism at the different stages of slaughtering in chicken processing plants and on how the processing procedure may affect such prevalence. The purpose of this study was to estimate the prevalence of *C. jejuni* at different stages of slaughtering in two California chicken processing plants.

MATERIALS AND METHODS

Sampling procedures. Two federally inspected California chicken processing plants cooperated in this study. Conventional slaughtering and processing techniques are used in both plants. Plant A is part of a fully integrated operation with its own source of chickens, and Plant B obtains chickens from several sources. Plant A uses three slaughtering procedures based on bird age and scald water temperature. Plant B has one procedure for all birds. Chlorinated city water is used in plant A, and plant B adds an additional 11 to 12 ppm of chlorine (11 to 12 μg/ml) to the water.

Sample sites were as follows: feathers from live hanging birds, scald water overflow, feather picker drip water, recycled water for cleaning gutters in the receiving room, ceca from the evisceration line, water from the final carcass wash, neck skin before chilling tank (NSBC), chiller water overflow, neck skin after chilling tank (NSAC), hearts and livers from giblet chiller, and wings ready for packaging. Sterile 18-oz (532 ml) Whirl-Pak bags (Nasco, Ft. Atkinson, Wis.) were used to collect water samples. Water drippings from at least five consecutive carcasses were obtained for each sample of water from the final carcass wash. Several crystals of sodium thiosulfate were added to each water sample to neutralize any residual chlorine. Five consecutive birds were sampled for each feather sample. Feathers were plucked from the breast region. Individual tissue samples were collected aseptically and placed in Whirl-Pak bags. All samples were placed in an ice chest, brought to the laboratory, and analyzed within 5 h after collection.

Media. A nutrient broth consisting of 20 g of polypeptone (BBL Microbiology Systems, Cockeysville, Md.), 2 g of yeast extract, 5 g of sodium chloride, and 1

liter of distilled water was used to wash the feathers, cecal contents, and tissue samples. The pH of the broth was adjusted to 7.4, and after autoclaving and cooling, the following antibiotics were added (per liter): 10 mg of vancomycin, 5 mg of trimethoprim lactate, and 5,000 IU of polymyxin B sulfate. Plating medium consisted of 52 g of brain heart infusion agar (Difco Laboratories, Detroit, Mich.), 0.5 g of yeast extract, 50 ml of lysed cow erythrocytes, 1 liter of distilled water, and the same concentrations of antibiotics used in the nutrient broth. The blood and antibiotics were added after the pH had been adjusted to 7.4 with 1 N NaOH and the medium had been autoclaved and cooled to 50°C.

Sample preparation. All water samples were plated directly onto the blood agar medium. Cecal contents were placed in sterile tubes, weighed, and agitated for 2 min in nutrient broth. The tubes were then centrifuged at 1000 × *g* for 5 min. Appropriate dilutions of the supernatant were plated, and the results are reported as the number of CFU per gram. Each feather and tissue sample was hand massaged and washed in the Whirl-Pak bag with nutrient broth for approximately 2 min before being plated at appropriate dilutions. Results for liver, wing, and heart samples are reported as the number of CFU per organ, and results for feather and neck skin samples are reported as the number of CFU per gram. Plates were incubated at 42°C for 48 h in anaerobic jars containing 5% CO_2, 10% O_2, and 85% N_2.

Identification of the organism. Smears of suspect colonies were examined by phase-contrast microscopy for typical morphology and motility. Suspect colonies were further characterized by the following biochemical tests: oxidase and catalase production, nitrate and sodium selenite reduction, H_2S production, and failure to grow in 3.5% sodium chloride (23).

Statistical methods. The data were analyzed by the Fisher exact probability test (18), the test for equality of two proportions, in which methods based on the normal approximation to the binomial distribution are used (15), and analysis of variance for a repeated-measures model (BMDP2V) (10).

RESULTS AND DISCUSSION

A total of 138 samples were obtained for each of four groups formed on the basis of bird age, scald water temperature, and plant sampled. Sampling was done on six mornings and included 11 lots of birds. Table 1 shows the *C. jejuni* isolation rates classified by group and sampling site, and Table 2 shows mean counts and ranges.

Breast feathers were sampled and analyzed as an indicator of external contamination of the birds with *C. jejuni*. Because the organism was not present or was present in such low numbers in most of the feather samples (Table 1), we suspected that the growth of other microbes on blood agar was suppressing *C. jejuni* growth. When the organism was present, the counts were quite high (Table 2).

Isolation rates for scald water overflow indicated a significant difference between groups 1 and 2 ($P < 0.05$). The difference may have been due to a combination of elevated water temperature and fewer birds with external contamination in group 1. Doyle and Roman (8) determined that the time required to kill 90% of the *C. jejuni* cells (*D*-value) at 55°C (131°F) ranged from 0.74 to 1.00 min. Since the birds are in the scald water for 90 s, the organism could survive the scald water of groups 2, 3, and 4. However, group 3, which had the lowest scald water temperature, had the lowest overall site isolation rate—55.1% (Table 1).

C. jejuni was isolated from 94.4% of the feather picker drip water samples, and the numbers of organisms present were high. This is an area where cross-contamination may occur, since the rubber finger-like projections that beat the feathers from the bird become contaminated and may pass the organism from bird to bird. However, the water used in rinsing the birds in the feather picker may physically remove the organism and thus reduce the number of organisms on the edible parts.

C. jejuni was recovered from every recycled water sample obtained from all four groups. This is understandable, since this water is recovered from the entire processing procedure, filtered to remove particulate matter, and used to wash waste material from the gutters in the receiving room (scalding and feather picking area). The use of recycled water to clean the gutters may further contaminate the receiving room with *C. jejuni* and potentiate widespread distribution of the organism within the plant. This distribution may occur through unnecessary movement of plant personnel from the receiving room to other areas of the plant.

The organism was recovered from 60 to 100% of the ceca in the four groups, and some numbers exceeded 10^6/g. This is consistent with the findings of others (9, 19). The isolation rate for the cecal contents of 3 of 12 lots birds was 0%, whereas that for the cecal contents of 6 lots was 100%. This, of course, indicates that there is a considerable degree of variability among lots of birds in carrier status. The isolation rate for group 4 differed significantly from those of the other groups ($P < 0.05$).

After evisceration, the birds pass through the final carcass wash, which is responsible for decreasing the number of organisms that contaminate the bird during evisceration. However, it is also possible for the water from the final carcass wash to trap the organism within the abdominal cavity or skin pockets.

There were significant differences among the NSBC sites of groups 2, 3, and 4. Group 1 differed from group 4, but the difference was not statistically significant and was probably due not to plant differences but to the fact that the overall rate at which the organism was recov-

TABLE 1. Isolation rates for *C. jejuni* obtained from different sampling sites during slaughtering in two California chicken plants

Group	Plant	Lot	Date (1982)	Scald water temp (°C)	Bird age (wk)	No. of isolations/total no. sampled												% Positive (all sites)
						Feathers	SWO[a]	FPDW[b]	RWCG[c]	Ceca	FCWW[d]	NSBC	CWO[e]	NSAC	Hearts	Livers	Wings	
1	A	1	6/4	60°C	12	1/5	0/3	3/3	3/3	5/5	3/3	5/5	3/3	5/5	5/5	3/3	3/3	
	A	2	6/18	60°C	12	0/5	0/3	3/3	3/3	0/5	3/3	4/5	3/3	3/5	1/5	3/3	3/3	
	A	3	6/18	60°C	12	1/5	0/3	3/3	3/3	4/5	2/3	2/5	3/3	4/5	2/5	0/3	3/3	
% Positive						13.3	0.0	100	100	60.0	88.9	73.3	100	80.0	53.3	66.7	100	66.7
2	A	4	6/4	53°C	7–8	1/5	3/3	3/3	3/3	5/5	3/3	5/5	3/3	5/5	5/5	3/3	3/3	
	A	5	6/15	53°C	7–8	0/5	0/3	3/3	3/3	0/5	0/3	0/5	3/3	4/5	3/5	0/3	0/3	
	A	6	6/24	53°C	7–8	3/5	2/3	3/3	3/3	5/5	3/3	5/5	3/3	5/5	4/5	3/3	3/3	
% Positive						26.7	55.6	100	100	66.7	66.7	66.7	100	93.3	80.0	66.7	66.7	75.8
3	A	5	6/15	49°C	7–8	0/5	0/3	1/3	3/3	0/5	0/3	0/5	3/3	0/5	0/5	0/3	0/3	
	A	7	6/22	49°C	7–8	3/5	0/3	3/3	3/3	5/5	3/3	5/5	3/3	5/5	1/5	3/3	3/3	
	A	8	6/29	49°C	7–8	0/5	3/3	3/3	3/3	4/5	3/3	5/5	3/3	4/5	1/5	1/3	2/3	
% Positive						20.0	33.3	77.8	100	60.0	66.7	66.7	100	60.0	13.3	44.4	55.6	55.1
4	B	9	6/22	53°C	7–8	0/5	0/3	3/3	3/3	5/5	3/3	5/5	3/3	3/5	3/5	3/3	1/3	
	B	10	6/24	53°C	7–8	0/5	0/3	3/3	3/3	5/5	3/3	5/5	3/3	5/5	4/5	3/3	0/3	
	B	11	6/29	53°C	7–8	2/5	2/3	3/3	3/3	5/5	3/3	5/5	3/3	5/5	5/5	3/3	3/3	
% Positive						13.3	22.2	100	100	100	100	100	100	86.7	80.0	100	44.4	78.3
% Positive (all groups)						18.3	27.8	94.4	100	71.7	80.6	76.7	100	80.0	56.7	69.4	66.7	68.1

[a] SWO, Scald water overflow.
[b] FPDW, Feather picker drip water.
[c] RWCG, Recycled water for cleaning gutters.
[d] FCWW, Water from final carcass wash.
[e] CWO, Chiller water overflow.

TABLE 2. Mean counts and range (log_{10}) for *C. jejuni* isolated from sampling sites in two California chicken plants

Group (plant)	Scald water temp (°C)	Bird age (wk)	Log_{10} mean count (range)[a]										
			Feathers[b]	SWO[c]	FPDW[c]	RWCG[c]	Ceca[d]	FCWW[c]	NSBC[c]	CWO[c]	NSAC[c]	Heart[f]	Liver[g]	Wing[g]
1 (A)	60	12	4.89 (4.56–5.22)	<1.00 (<1.00)	4.17 (3.11–5.00)	3.07 (2.29–4.15)	6.23 (4.10–7.01)	2.85 (2.00–4.20)	2.62 (1.00–4.23)	1.92 (1.30–2.59)	1.38 (1.00–2.12)	2.10 (1.70–2.70)	2.93 (2.00–3.78)	2.81 (2.00–4.26)
2 (A)	53	7–8	3.86 (2.56–4.94)	2.20 (1.48–2.84)	4.05 (2.60–5.00)	2.98 (1.30–4.08)	6.82 (5.85–7.11)	3.12 (2.10–3.78)	2.92 (2.39–4.51)	2.62 (2.00–3.40)	1.74 (1.00–2.80)	2.32 (1.70–2.95)	3.31 (2.81–3.48)	3.45 (3.11–3.70)
3 (A)	49	7–8	4.19 (2.80–5.04)	1.43 (1.00–2.00)	3.25 (1.48–3.86)	2.43 (1.00–3.34)	5.56 (3.72–6.68)	2.60 (1.84–3.28)	3.32 (2.37–4.34)	1.74 (1.00–2.10)	1.33 (1.00–2.26)	1.85 (1.70–2.00)	2.49 (2.00–3.16)	3.27 (3.00–3.58)
4 (B)	53	7–8	4.75 (4.25–5.25)	2.15 (2.00–2.30)	4.04 (3.51–4.41)	4.14 (3.84–4.41)	6.49 (4.91–7.25)	2.57 (1.30–4.30)	2.50 (1.28–3.76)	2.52 (1.60–2.95)	1.76 (1.00–2.63)	2.23 (1.70–2.51)	3.25 (2.48–4.15)	2.85 (2.00–3.31)

[a] Abbreviations are explained in Table 1 footnotes.
[b] Detection level, Log_{10} 2 CFU/g.
[c] Detection level, Log_{10} 1 CFU/ml.
[d] Detection level, Log_{10} 3 CFU/g.
[e] Detection level, Log_{10} 1 CFU/g.
[f] Detection level, Log_{10} 1.70 CFU per organ.
[g] Detection level, Log_{10} 2 CFU per organ.

ered from the ceca of groups 1, 2, and 3 was lower than that for group 4. The results for group 1, lot 2, (Table 1), in which the organism was not isolated from the ceca but was recovered from the NSBC, present a contradiction. Sampling chance alone may have accounted for this, or the organism may have been present in the ceca at numbers lower than the detection level and subsequently contaminate the NSBC.

After passing through the final wash, the carcasses enter a water chilling tank maintained at 0 to 1°C (33 to 34°F). In this study, the organism was recovered from all of the chiller water overflow samples. According to these findings, the chilling tank represents a major area where cross-contamination may occur. Carcasses that enter the chilling tank free of the organism may become contaminated in the tank, whereas heavily contaminated carcasses leave the tank with fewer organisms. Table 2 shows how the NSAC counts were always lower than the NSBC counts. However, the overall prevalence of the organism on NSAC was slightly higher than that on NSBC. *C. jejuni* survived the chlorinated water used in plant B. Other investigators have also reported that *C. jejuni* has been recovered from poultry that had been processed in plants using chlorine (13).

The NSAC, hearts, livers, and wings were sampled as ready-for-market products. The overall isolation rate for the four groups was 68%. This is within the range reported by others (5, 9, 14, 17, 22). The isolation rates for group 3 hearts were significantly different from those for hearts from groups 2 and 4 ($P < 0.05$). This is probably due to the fact that group 3 samples were obtained in the early morning, before many birds had passed through the plant. With fewer birds passing through the plant, there is less chance of contamination.

By the Scheffes contrast method (11), we determined that the mean rate of isolations for the scald water overflow was significantly lower ($P < 0.05$) than the corresponding mean rates for recycled water used to clean gutters, chiller water overflow, water used for the final carcass wash, and feather picker drip water.

Overall group prevalence comparisons revealed significant differences ($P < 0.05$) between groups 1 and 3, 1 and 4, 2 and 3, and 3 and 4. Comparisons of groups 1 and 2 and groups 2 and 4 showed nonsignificant results.

The two factors for the analysis of variance for repeated measures were group and site. This analytical method made use of the replication in each group-site combination. The three analyses were as follows: all sampling sites, water sampling sites, and sites where products were ready for market. In all three analyses, a significant group-site interaction was not observed.

Overall, this study illustrates the apparent variability among lots of birds in presence of *C. jejuni*. Some lots of birds harbor the organism in the ceca, and carcasses are contaminated during processing, whereas other lots are devoid of *C. jejuni*. A study conducted at the farm level may identify a factor(s) responsible for the levels of the organism carried by birds. This aspect of *C. jejuni* ecology is now under study.

With respect to food safety, the significance of the presence and numbers of *C. jejuni* in ready-for-market poultry meat cannot be critically assessed since we do not presently know what a pathogenic campylobacter is (1, 4, 7). Available reports (1) indicate that poultry meat has been implicated epidemiologically only in very few outbreaks of campylobacteriosis. Heterogeneity within the *Campylobacter* group has been demonstrated (21). In practical terms, this may mean that not all *C. jejuni* strains found in poultry are pathogenic.

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